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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00020-19

PERIOD COVERED

October 01, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies with PolyICLC

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Hilton B. Levy, Ph.D., Special Assistant to the Scientific Director, NIAID

COOPERATING UNITS *(if any)*

Dr. Andres Salazar, Walter Reed; Dr. Meir Kende, USAMRIID; Dr. Radha Meheshwari, USUHS; Dr. G. Dutta, Central Drug Research Institute, Lucknow, India

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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies using PolyICLC in the treatment of patients with glioma continues. We now have 38 patients on study long enough for evaluation. There are 14 anaplastic astrocytomas (Grade 3); 18 glioblastoma neoformans (Grade 4); and 6 recurrent gliomas. Based on historical controls, the Grade 3's have a median life expectancy after diagnosis of 18 months. All of our treated Grade 3's are alive and essentially symptom-free 37 months after diagnosis. Their tumors mostly continue to shrink as measured by gadolinium enhanced M.R.I. The Grade 4's, the most malignant type, as expected are not doing that well, but PolyICLC does appear to produce some increase in useful life.

The multiple sclerosis patients continue to be stable. The studies with malaria in India continue. We have found disease caused by strains of malaria that have become resistant to chloroquine can be controlled by adding PolyICLC to the treatment. PolyICLC also increases the rate of wound healing in rats and monkeys. Patients have been filed for the use of PolyICLC in control of malaria and in wound healing.

PHS-NIH
Summary Report
Molecular Allergy and Immunology Section
Jean-Pierre Kinet, M.D., Section's Head
October 1, 1993 through September 30, 1994

Introduction

One of the major goal of the Molecular Allergy and Immunology Section is to understand the molecular mechanisms which regulate the initiation of allergic diseases. The section has contributed in a major way in the understanding of the structure and function of the high affinity IgE receptor (FcεRI), one of the key molecule involved in the initiation of allergic reactions. MAIS scientists have made important progress in understanding of how FcεRI binds its ligand IgE and how FcεRI signals into the cells. Moreover they demonstrated that by targetting FcεRI, one should be able to prevent allergic reactions.

The role of glycosylation in the folding and secretion of human truncated FcεRI α chain.

The high affinity immunoglobulin E receptor (FcεRI) is a $\alpha\beta\gamma_2$ tetrameric complex. The α chain, which is heavily glycosylated, binds immunoglobulin E (IgE) while the β and γ chains are involved in signal transduction. The truncated extracellular segment of the α chain (αt) is sufficient for high affinity binding of IgE and is secreted in active form when expressed by transfection in eukaryotic cells. MAIS scientists have analyzed the role of glycosylation in the folding, intracellular transport and secretion of αt. They found that the maturation of N-linked oligosaccharides and the addition of O-linked carbohydrates, both of which occur in the Golgi apparatus, are not required for either transport or the IgE-

binding function of αt. Furthermore, the binding function is not affected negatively by enzymatic deglycosylation. However, analysis of a panel of mutants shows that the seven putative N-linked glycosylation sites are all glycosylated and have a cumulative effect on the proper folding and secretion of αt. The majority of αt remains unfolded when glycosylation of αt is abolished by mutagenesis of the seven sites or by tunicamycin treatment of the transfected cells. Therefore, the multiple glycosylations of the α chain are necessary for its efficient folding in the endoplasmic reticulum of eukaryotic cells.

Differential control of the tyrosine kinases lyn and syk by the two signaling chains of the high affinity immunoglobulin E receptor.

Non-receptor tyrosine kinases such as the newly described 70 kDa (ZAP-70/syk) and src related tyrosine kinases are coupled to a variety of receptors including the antigen receptors on B- and T-cells and the Fc receptors for IgE (FcεRI) and IgG (FcγRI, FcγRIII/CD16). Various subunits of these receptors contain homologous activation motifs which appear capable of autonomously triggering cell activation. Two forms of this motif are present in the FcεRI multimeric complex: one in the β chain and one in the γ chain. MAIS scientists have shown that each of the two tyrosine kinases known to be involved in FcεRI signaling is controlled by a distinct motif-containing chain. Lyn associates with the non-activated β chain while γ promotes the activation of syk. We also show that neither the β nor the γ motif alone can account for the full signaling capacity of the entire

receptor. They found that, upon triggering of the tetrameric receptor, lyn already bound to β becomes activated and phosphorylates β and γ; the phosphorylation of γ induces the association of syk with γ and also the activation of syk, resulting in the phosphorylation and activation of $PLC\gamma_1$. Cooperative recruitment of specific kinases by the various signaling chains found in this family of antigen receptors could represent a way to achieve the full signaling capacity of the multimeric complexes.

Abolition of anaphylaxis by targeted disruption of the high affinity IgE receptor α chain gene.

Mast cells and basophils, which are activated by IgE and allergen, play a prominent role in anaphylaxis. However, they express at least three types of IgE receptor, including the high affinity IgE receptor (FcεRI). The relative contribution of these, and possibly other receptors such as CD23/FcεRII and Mac-2, to the genesis of *in vivo* anaphylaxis is still unclear. To address this question, MAIS scientists have generated FcεRI-deficient mice. These mice appear normal and express a normal number of mast cells, but they are resistant to cutaneous and systemic anaphylaxis. These data demonstrate that FcεRI is necessary for the initiation of IgE-dependent anaphylactic reactions. Therefore, interfering with its function should be an effective means of treating allergy, regardless of the allergen specificity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00664-03 ODIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

Distribution and role of FcεRI in human cells

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

K. Ochiai	Visiting Fellow	MAIS, NIAID
M-H. Jouvin	Visiting Associate	MAIS, NIAID
D. Dombrowicz	Visiting Fellow	MAIS, NIAID
V. Flamand	Special Volunteer	MAIS, NIAID

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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK

In rodents, FcεRI has only been detected so far on the surface of mast cells, basophils, and non-B non-T cells. The role played by this receptor in initiating cell degranulation and the release of the mediators of allergic reactions is clearly established.

In humans however, the cell distribution of FcεRI appears to be different. We found last year that human Langerhans cells from the skin of normal individuals also express FcεRI. More recently, we also found that dermal dendritic cells express FcεRI as well. In addition, monocytes of allergic individuals also express this receptor. We are attempting to define the factors which regulate expression of FcεRI on these cells and the role of this receptor in this new context. As a way to further our understanding of these questions, we are also generating transgenic mice overexpressing human alpha chains under the control of the human promotor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00662-03 ODIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

Characterization of the FcεRI α chain by crystallography

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

Jami W. Brown	Microbiologist	MAIS, NIAID
Salvatore Sechi	Visiting Associate	MAIS, NIAID

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK

The receptor with high affinity for IgE (FcεRI) is a tetrameric complex of an IgE binding α chain, a β chain and a dimer of γ chains. In the past we have found that the extracellular region of the α chain is solely responsible for ligand binding. In order to further characterize the receptor binding site, we use CHO cells to generate various transfectants secreting the extracellular domain of alpha with the hope of generating crystals for crystallographic analysis. The secreted alpha is active but the heterogenous glycosylation prevents crystallization. Deglycosylation by mutation of the seven N-linked glycosylation sites results in misfolding of the alpha chain in the endoplasmic reticulum of eukaryotic cells. For this reason, we have produced deglycosylated alpha chain in E-Coli. However, the deglycosylated alpha tends to aggregate and therefore is unsuitable for crystallization. Recently, by using limited deglycosylation, we have been able to produce a preparation of alpha chain which is crystallizing. The crystals difract at 4.5 angstrom and are therefore not good enough for standard crystallographic analysis. However, we hope that by adapting the conditions we will be able to produce better crystals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00665-03 ODIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

The role of the FcεRI receptor subunits and of the receptor associated molecules in signal transduction

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK

The high affinity IgE receptor belongs to a class of receptor which lacks intrinsic enzymatic activity but activates non-receptor tyrosine and serine/threonine and phosphatases. This activations leads to phosphorylation and activation of phophatidylinositol-specific phospholipase C, an increase in intracellular calcium and ultimately the cell degranulation and the release of the mediators of allergic reactions.

Last year we had identified a distinct role for the β and γ subunit: β but not γ associates with the tyrosine kinase lyn in resting conditions, and γ but not β can activate the tyrosine kinase syk. Based on our findings, we had proposed a model of FcεRI activation in which, upon receptor aggregation lyn phosphorylates both β and γ, and the phosphorylation of γ is necessary to recruit syk and activate syk. We now have tested this model of activation by reconstitution the various elements in fibroblasts. This is accomplished by infection of the fibroblasts expressing the tetrameric FcεRI receptor with recombinant vaccinia viruses. In this system, Lyn is the only kinase required for receptor phosphorylation. Cotransfection of Syk does not alter the phosphorylation state of FcεRI due to Lyn alone. The presence of the tetrameric receptor and Lyn is also required for the formation of a receptor-Lyn-Syk complex, which is increased after receptor aggregation. Thus, the cooperation between the two kinases and the β and γ subunits is necessary for the full capacity of the FcεRI receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00668-03 ODIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

Production of IgE receptor deficient mice by homologous recombination

PRINCIPAL INVESTIGATOR

PI: Jean-Pierre Kinet, Section Chief MAIS, NIAID

Others:

V. Flamand	Visiting Fellow	MAIS, NIAID
D. Dombrowicz	Visiting Fellow	MAIS, NIAID
M-H. Jouvin	Visiting Scientist	MAIS, NIAID

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Beverly Köller (The University of North Carolina)

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK

Mast cells and basophils, which are activated by immunoglobulin E (IgE) and allergen, play a prominent role in anaphylaxis. However, they express at least three types of IgE receptors: the high affinity IgE receptor (FcεRI), and the two low affinity IgG receptors (FcγRII and FcγRIII) also capable of binding IgE. The relative contribution of these, and possibly other receptors such as CD23/FcεRII and Mac-2, to the genesis of *in vivo* anaphylaxis is still unclear. To address this question, we have generated mice deficient mice with a disrupted FcεRI α chain gene. This defect results in complete suppression of the cell surface expression of FcεRI. These mice appear normal and express a normal number of mast cells, but they are resistant to cutaneous and systemic anaphylaxis. These data demonstrate that FcεRI is necessary for the initiation of IgE-dependent anaphylactic reactions. Therefore, interfering with its function should be an effective means of treating allergy, regardless of the allergen specificity.

We are also in the process of generating mice with a disrupted FcεRI β chain gene. Since the β chain not only associate with FcεRI but also with FcγRIII, these mice should allow us to analyze the contribution of the β chain in FcγRIII-related cell activation.

Laboratory of Molecular Structure
1994 Annual Report
Table of Contents

Z01-AI Project Number		Page
Annual Report		15-1
00169-17	Analysis of Murine and Human Transplantation Antigens and Genes -- Coligan	15-8
00172-16	Synthesis of Peptide Antigens -- Coligan	15-9
00352-12	Characterization of Cell Surface Molecules Important for Immune Function -- Coligan	15-10
00522-07	Protein Sequence Analyses -- Coligan	15-11
00523-07	Flow Cytometric Analysis -- Holmes	15-12
00543-07	Recognition of Peptide Antigens by Virus-Specific Cytotoxic T Lymphocytes -- Coligan	15-13
00595-04	Purification, Characterization and Function of Proteins of the Complement System -- Hammer	15-14
00697-01	Structural Characterization of Proteins -- Sun	15-15

Annual Report
Laboratory of Molecular Structure
National Institute of Allergy and Infectious Diseases
October, 1, 1993 to September 30, 1994

RESEARCH PROGRESS

The Lab of Molecular Structure (LMS) investigates the structure and function of the genes and molecules involved in the immune response, the molecular basis for antigen presentation and recognition, and the molecular mechanisms controlling T-cell development. Special emphasis is placed on membrane receptors and differentiation antigens, in particular the family of adhesion molecules known as integrins, present on T and B lymphocytes, on the structures of major histocompatibility genes and proteins, on the nature of the peptides bound by class I and class II molecules, on the molecular basis by which antigens are processed and presented to T cells, on the *cis* elements and *trans* factors that regulate T cell receptor and class I gene expression and on proteins of the complement system. The recently created Structural Biology Section will concentrate on determining the molecular structure and function of immunologically relevant molecules, such as cytokines and their receptors, and T cell receptors. In addition to this fundamental immunological research, the LMS provides protein sequencing, peptide synthesis, mass spectrometric, and flow cytometric expertise and services for the DIR.

STUDIES ON THE MAJOR HISTOCOMPATIBILITY COMPLEX

Major histocompatibility complex (MHC) encoded class I molecules are integrally involved in the presentation of intracellular proteins such as viral antigens, in the form of processed peptides, to cytotoxic T lymphocytes (CTL). Studies on the molecular basis of viral antigen processing and the nature of the interaction of processed antigen (peptides) with class I molecules and T cell receptors are emphasized in this research.

Peptide Binding Motifs Can Be Used to Predict CTL Epitopes. The potential value of peptide binding motifs of HLA class I molecules for the prediction of viral epitopes presented to T cells has been analyzed for HLA-A1 and -A3. CTL generated against type A influenza virus recognize epitopes derived from the nucleoprotein (NP) and basic polymerase 1 presented by HLA-A1, and epitopes derived from NP presented by HLA-A3. Distinct peptide binding motifs with characteristic anchor residues were previously identified for each of these class 1 molecules based on the sequences of endogenous peptides. Peptides containing the either HLA-A1 or -A3 binding motifs were identified within the sequences of the NP and basic polymerase 1 proteins. Two of the HLA-A1 binding peptides could sensitize target cells for lysis by influenza virus-immune CTL populations restricted by HLA-A1, and the one HLA-A3 NP peptide could sensitize target cells for lysis by HLA-A3 restricted influenza-immune CTL. Each peptide was also shown to be able to induce peptide-specific class I-restricted CTL in vitro, and the CTL generated against two of these peptides could specifically recognize virus-infected targets. Thus, these peptide binding motifs can be used to construct immunogenic synthetic epitopes which are capable of inducing antiviral T cell-mediated immune responses.

Sequence Motifs Important for Peptide Binding to HLA-A1 and HLA-B8. Distinct amino acid (aa) residue motifs for peptides binding to HLA-A1 and HLA-B8 were identified by sequence analyses of reversed-phase HPLC fractions containing endogenous peptides derived from these HLA molecules. Common features among the peptide HLA-A1 sequences were Tyr at the COOH-terminus, a negatively charged aa (usually Glu) at position 3 (P3), and pro at P4. Twenty-seven different primary sequence assignments were made for HLA-B8 associated peptides, most of which were eight aa in length. Lys, and in a few cases Arg, predominated at P3 and P5; Leu and Pro predominated at P2, and Leu was the preferred COOH-terminal residue. Unlike all other human class I molecules whose peptide-binding properties have been studied, both HLA-A1 and HLA-B8 endogenous peptide sequences have a dominant anchor residue at P3, and these aa are opposite in charge to the aa position at position 156 of the HLA peptide-binding site. The HLA-A1 peptide-binding motif was then used to identify potential antigenic peptides from influenza A viral proteins that bound to HLA-A1 in vitro.

Method for Predicting T Cell Epitopes for Vaccine Development. A method to predict the relative binding strengths of all possible nonapeptides to the MHC class I molecule HLA-A2 was developed based on experimental peptide binding data. These data indicate that, for most peptides, each side-chain of the peptide contributes a certain amount to the stability of the HLA-A2 complex that is independent of the sequence of the peptide. To quantify these contributions, the binding data from a set of 154 peptides were combined together to generate a table containing 180 coefficients (20 amino acid x 9 positions), each of which represents the contribution of one particular amino acid residue at a specified position within the peptide to binding to HLA-A2. The coefficients were then used to calculate the theoretical binding stability for all previously identified self or antigenic nonamer peptides known to bind to HLA-A2. The binding stability for all other nonamer peptides that could be generated from the proteins from which these peptides were derived was also predicted. In every case, the previously described HLA-A2 binding peptides were ranked in the top 2% of all possible nonamers for each source protein. Therefore, most nonamer peptides relevant to cytotoxic T cell recognition should be identifiable using the table of coefficients. We have shown that the same predicative methodology is applicable to other class I molecules.

Regulatory Elements Important for Class I Expression. The MHC class I regulatory element (CRE) region 1 has been previously described as a positive *cis*-acting regulatory element essential for class I gene expression. We have generated transgenic mice with the MHC class I gene H-2D^d driven by two different 400-bp promoter regions of Q10, a nonpolymorphic MHC class I gene expressed in the liver, kidney, and fetal yolk sac. One construct (Q10M3/D^d) had 2 bp substitutions introduced in region 1 of the CRE that reconstituted the CRE inverted repeat present in classical class I genes. Mice containing the wild-type Q10/D^d gene expressed membrane-bound H-2D^d molecules in a tissue-restricted expression pattern similar to that observed for endogenous Q10. In mice containing the mutant construct (Q10M3/D^d), H-2D^d was also expressed in the thymus, a tissue not normally associated with Q10 expression but, surprisingly, the D^d was not expressed in other lymphoid tissues. Thymic D^d expression was correlated with the presence of what appears to be a previously unidentified transcription factor. These results show that the mutations in region 1 altered the tissue-specific regulation of the Q10 promoter in-vivo, and that elements in addition to CRE region 1 in the Q10 promoter region serve to limit ubiquitous tissue expression.

ROLE OF ADHESION MOLECULES (INTEGRINS) IN THYMIC DEVELOPMENT

THYMOCYTE-MEROSIN INTERACTIONS PLAY A ROLE IN THYMIC DEVELOPMENT. Merosin is a 700 kDa multichain protein that has several properties in common with laminin, one of the major extracellular matrix proteins present in basement membranes. Both contain B1 and B2 light chains, but merosin has a distinct M-chain compared with the laminin A-chain. Using the RT-PCR assay, we demonstrate that merosin, but not laminin, is also expressed in the thymus. 18-24% of adult mouse thymocytes bound to merosin in vitro. Binding was inhibited by antibodies recognizing VLA α_6 or β_1, suggesting that the VLA-6 integrin is a merosin receptor. Virtually all thymocytes expressed VLA-6, but only immature thymocytes ($J11d^+$) bound to merosin. PMA treatment did not significantly increase the binding of immature thymocytes nor did it induce binding in the mature $J11d^-$ population. In contrast, both splenic T cells and unseparated lymph node cells showed enhanced binding to merosin after PMA stimulation. The expression of merosin in the thymus and its selective interaction with immature thymocytes suggest that thymocyte-merosin interactions may play a role in T cell development.

FIBRONECTIN RECEPTORS ARE IMPORTANT IN THYMIC DEVELOPMENT. We have analyzed the expression and function of $\alpha4\beta1$ fibronectin binding integrin receptors on human thymocytes. A major portion of double positive ($CD4^+CD8^+$) human thymocytes express $\alpha4\beta1$ in a constitutively active form and adhere to fibronectin and vascular cell adhesion molecule 1. $\alpha4\beta1$ expression is similar on adherent and nonadherent populations, thus, activity reflects the receptor state and not simple expression. These adherent cells are immature, whereas nonadherent cells possess the phenotype of thymocytes after positive selection. The adherent population fails to respond to activation with anti-CD3 and fibronectin, whereas nonadherents exhibit an $\alpha5\beta1$-dependent proliferation. Thus, differential regulation of $\alpha4\beta1$ and $\alpha5\beta1$ integrin receptors for fibronectin may provide a mechanism for controlling cellular traffic, differentiation, and positive selection of thymocytes.

PROTEINS OF THE COMPLEMENT SYSTEM

We have identified a unique C4-binding plasma sialoglycoprotein with a molecular weight of 120 kDa (sgp 120) that has the capacity to regulate classical complement function *in vitro*. The biologic role of this protein and cleavage peptides may be learned from the recent elucidation of its gene structure which codes for several expressed variants, the largest containing 893 amino acids including a 35 residue insert. Experimental data indicates that Sgp 120 is an acute phase protein with 76% homology to the acute phase pig protein, Pigtryin and as much as 51% identity (HC3) to the members of Inter-alpha Trypsin Inhibitor family (IATI) and may share structural and functional properties here as well.

NEWLY ESTABLISHED STRUCTURAL BIOLOGY PROGRAM

The setting up of the Structural Biology Section began with acquiring all of the necessary equipment for each of three areas of the section; X-ray diffraction, computer support and protein chemistry. Projects currently in progress are i) crystallization and structure determination of TGF-β receptor, TGF-β1 LAP and their complexes; ii) crystallographic studies of cytokines, like CTGF (a connective tissue growth factor) and IL-13; iii) expression, purification and crystallization of immune related receptors, including type 1 interferon receptor and T cell receptor iv) structure determination of a bioluminescent protein aequorin.

SYNTHESIS OF PEPTIDE ANTIGENS

During the past year, almost 700 peptides have been produced by the Laboratory. Many have

been used for defining MHC class I restricted T cell epitopes and for defining the nature of the interaction between peptides and class I molecules. Peptides have also been extensively used for studying MHC class II restricted epitopes and preparing antisera for a large variety of proteins including several B cell specific proteins, human histamine I receptor, membrane associated protein of *Crithidia fasciaulata, P. falciparum* protein involved in red blood cell invasion, phosphoprotein signaling domains, the vasodilator maxadilan, HIV-Tax, IκB, various vaccinia proteins such as complement control protein, membrane associated protein of *Toxoplasma gondii*, purinergic receptor, T cell receptors, PLC gamma

1 and 2 enzymes, various protein kinases, DNA replication protein of Epstein-Barr virus, actin I and II of *P. falciparum*, SIV proteins, rabbit RAG-2 and human FCεR1 β and γ chains. Peptides have also been used to study the biological activity of ring-finger DNA proteins, HIV proteins (Tat, Rev and TAR), Duffy blood group antigens, a malaria parasite anti-oxidant; an apoptosis associated ceramide-activated threonine protein kinase, and IL1 β converting enzyme. Peptides have also been used extensively to study T helper and CTL recognition of peptides associated with HIV, toxoplasmosis, myelin basic protein (multiple sclerosis associated), Friend virus, influenza virus, and a large variety of autoimmune disease such as arthritis, gastritis, EAE and myasthenia gravis.

Synthetic peptides representing the immunodominant epitopes of $p40^{-Taxl}$ were used to develop an enzyme immunoassay that was specific for detecting HTLV-I anti-tax antibodies and thus can be used to distinguish HTLV-I and HTLV-II infections. Use of a similar peptide based assay showed that patients with HTLV-I-associated myelopathy (HAM) or adult T cell leukemia (ATL) could be distinguished by their differential immune responsiveness to the immunodominant epitopes of the tax and rex proteins suggesting that such responses may relate to pathogenesis. Finally, an extended tax protein that appears to be a unique characteristic of most HTLV-IIb isolates was found to be useful for an immunoassay that could distinguish HTLV-IIa and HTLV-IIb infections.

PROTEIN ANALYSES

The LMS determined the amino acid sequences of about 650 samples. About 440 of these analyses were for unknown protein or peptides and the remainder were for quality control of synthetic peptides. Most of the unknown sequences determined (about 250) were of endogenous peptides eluted from mouse d and b haplotype class I molecules (LI/NIAID, D. Margulies) and from human HLA-A3, -B44, -B14, -B8 and -A1 molecules (LMS). From these analyses, amino acid residues in peptides requisite for peptide binding to particular class I molecules have been determined. This information has been used to search protein sequence databanks for potential antigenic T cell epitopes that are recognized by cytotoxic T lymphocytes which has important implications for vaccine development. Using such an approach several new antigenic epitopes recognized by cytotoxic T lymphocytes specific for influenza virus proteins and myelin basic protein have been identified. In addition, proteins and peptides from a large variety of other sources have been sequenced by the facility.

A disulfide-linked heterodimeric antigen from human B leukemia cells was detected by radioimmunoprecipitation and Western blot analysis using a monoclonal antibody generated against a cell membrane antigen preparation from human B prolymphocytic leukemia cells and found to define an extracellular epitope of the smaller component (β chain) of a heterodimeric antigen on human B leukemia cells. Determination of the amino terminal amino acid sequences of the α and β chains unequivocally identified them as the human mb-1 and B29 proteins, respectively. A β-tubulin-like protein was found to co-purify with the mb-1-B29 antigen and we found that there is a strong amino acid sequence homology between the microtubule-binding domains of certain human microtubule-associated proteins and an intracellular segment of the human mb-1 protein.

FLOW CYTOMETRIC ANALYSES

The amount of usage by DIR labs has increased 3539 hrs in FY 93 to 3659 hrs in FY 94. The percentage of this time devoted to sorting has increased from 48% to 56% of total usage. Therefore, for the first time, more than half of the usage time (including use of the user operated Epics Profile) was devoted to sorting.

Administrative Report

This year marked the creation of the *Laboratory of Molecular Structure* which consists of the previous Biological Resources Branch and a newly established Structural Biology research component.

The *Molecular Immunology Section* saw the addition of Dr. Andrew Brooks from the Flinders University of South Australia, of and Dr. Huaxian Chen, from the Department of Microbiology, University of Kansas Medical Center, and Dr. Stephanie Otto departed to assume a position in government affairs.

In the *Biological Resources Section*, the *Protein Analysis and Synthesis Unit* maintained production by synthesizing over 700 peptides and sequencing about 650 peptides/proteins. The facility upgraded its existing Vestec lasar assisted matrix desorption (MALDI) - mass spectrometer by adding a reflectron for greater resolution and a higher energy lasar for increased sensitivity. Two new Applied Biosystems Inc. protein sequencers were also acquired along with a Rainin Symphony multiple peptide Synthesizer. Michael Knierman left the facility for personal reasons. The *Flow Cytometry Unit* installed a Facscan and has purchased a FACs Vantage multiparameter flow cytometer, and the FACstar plus was upgraded to perform simultaneous 5 color analyses.

A new *Structural Biology Section* was established and Dr. Peter Sun was hired as a tenure track investigator and Dr. Linda Hannick was hired to provide technical support for this operation as well as to conduct original scientific research. Dr. Maria Cueto of Merck & Co. was hired as a postdoctoral fellow. Extensive equipment to support this facility was acquired. X-ray diffraction units consisting of two Rigaku RU200 rotating anode X-ray generators as X-ray sources, two RAXIS-II imaging plate systems as 3-D diffraction data collection devices and an Enraf-Nonius precession camera for 2-D diffraction data collection. The computing support has four DEC Alpha 3000/800 series of workstations for speed computation, five Silicon Graphics Indigo 2-extreme workstations for computation and graphics interface, one Evans and Sutherland workstation as graphics interface two PC-486 computers and one MAC for simple graphics and word processing.

Honors and Awards

Dr. Coligan was invited to present research seminars at the Center for Disease Control in Atlanta, the University of Leiden, the Netherlands, Department of Microbiology and Immunology, Uniformed Services Medical School, and the Roswell Park Memorial Cancer Institute. Dr. Coligan made invited presentations at the Keystone Symposium on T and B lymphocytes, the NIAID Rocky Mountain Laboratory Immunology Symposium, and the H2/HLA Workshop. Dr. Coligan continues to serve on the editorial boards of Current Protocols in Immunology, Molecular Immunology, and Immunologic Research, and was appointed as an editor for Current Protocols in Protein Science and the Journal of Biomedical Science. Dr. Coligan serves on the Immunology and Immunotherapy Study Section of the American Cancer Society and the Board of the Structural Biology Interest Group at the NIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00169-17 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Chief, LMS/NIAID

Others:

K. Parker	Senior Staff Fellow	LMS/NIAID	J. Shuman Senior Staff Fellow	LMS/NIAID
A. Brooks	Visiting Fellow	LMS/NIAID		
Y. Shirakata	Visiting Associate	LMS/NIAID		
H. Chen	IRTA	LMS/NIAID		

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.4	3.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The MHC class I regulatory element (CRE) region 1 has been previously described as a positive *cis*-acting regulatory element essential for class I gene expression. We have generated transgenic mice (CBA x C57BL/6) with the MHC class I gene H-2D^d driven by the 400-bp promoter region of Q10, a nonpolymorphic MHC class I gene expressed in the liver, kidney, and fetal yolk sac, that had 2 bp substitutions introduced in region 1 of the CRE that reconstituted the CRE inverted repeat present in classical class I genes. In mice containing the mutant construct (Q10M3/D^d), H-2D^d was also expressed in the thymus, a tissue not normally associated with Q10 expression but, surprisingly, the D^d was not expressed in other lymphoid tissues. Furthermore, thymic expression was greatest on double positive (CD4$^+$ CD8$^+$) thymocytes. Thymic D^d expression was correlated with the presence of what appears to be a previously unidentified transcription factor in thymocytes that is capable of interacting with the CRE-inverted repeat. These results show that the mutations in region 1 altered the tissue-specific regulation of the Q10 promoter in-vivo, although an intact inverted repeat did not restore the ubiquitous pattern of expression characteristic of classical class I genes. Thus, these results indicate the elements in addition to CRE region 1 in the Q10 promoter region serve to limit ubiquitous tissue expression.

A method to predict the relative binding strengths of all possible nonapeptides to the MHC class I molecule HLA-A2 has been developed based on experimental peptide binding data. These data indicate that, for most peptides, each side-chain of the peptide contributes a certain amount to the stability of the HLA-A2 complex that is independent of the sequence of the peptide. To quantify these contributions, the binding data from a set of 154 peptides were combined together to generate a table containing 180 coefficients (20 amino acid x 9 positions), each of which represents the contribution of one particular amino acid residue at a specified position within the peptide to binding to HLA-A2. The "theoretical" binding stability (calculated by multiplying together the corresponding coefficients) matched the experimental binding stability to within a factor of 5. The coefficients were then used to calculate the theoretical binding stability for all previously identified self or antigenic nonamer peptides known to bind to HLA-A2. The binding stability for all other nonamer peptides that could be generated from the proteins from which these peptides were derived was also predicted. In every case, the previously described HLA-A2 binding peptides were ranked in the top 2% of all possible nonamers for each source protein. We conclude that the side-chains of most nonamer peptides to the first approximation bind independently of one another to the HLA-A2 molecule and that the methodology that we have developed can be used to predict T cell epitopes important for vaccine development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00172-16 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis of Peptide Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Chief, LMS, NIAID

Others:

Carl Hammer, Ph.D., Senior Investigator, LMS, NIAID

Jan Lukszo, Ph.D., Senior Staff Fellow, LMS, NIAID

COOPERATING UNITS *(if any)*

Centers for Disease Control (Dr. Renu Lal); NICHHD (Dr. K. Ozato); USHHS (Dr. S. Vogel)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Biological Resources Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	1.7	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year, almost 700 peptides have been produced by the Laboratory. Many have been used for defining MHC class I restricted T cell epitopes and for defining the nature of the interaction between peptides and class I molecules. Peptides have also been extensively used for studying MHC class II restricted epitopes and preparing antisera for a large variety of proteins including several B cell specific proteins, human histamine I receptor, membrane associated protein of *Crithidia fasciaulata, P. falciparum* protein involved in red blood cell invasion, phosphoprotein signaling domains, the vasodilator maxadilan, HIV-Tax, IκB, various vaccinia proteins such as complement control protein, membrane associated protein of *Toxoplasma gondii*, purinergic receptor, T cell receptors, PLC gamma 1 and 2 enzymes, various protein kinases, DNA replication protein of Epstein-Barr virus, actin I and II of *P. falciparum*, SIV proteins, rabbit RAG-2 and human FCεR1 β and γ chains. Peptides have also been used to study the biological activity of ring-finger DNA proteins, HIV proteins (Tat, Rev and TAR), Duffy blood group antigens, a malaria parasite anti-oxidant; an apoptosis associated ceramide-activated threonine protein kinase, and IL1 β converting enzyme. Peptides have also been used extensively to study T helper and CTL recognition of peptides associated with HIV, toxoplasmosis, myelin basic protein (multiple sclerosis associated), Friend virus, influenza virus, and a large variety of autoimmune disease such as arthritis, gastritis, EAE and myasthenia gravis.

Synthetic peptides representing the immunodominant epitopes of $p40^{-TaxI}$ were used to develop an enzyme immunoassay that was specific for detecting HTLV-I anti-tax antibodies and thus can be used to distinguish HTLV-I and HTLV-II infections. Use of a similar peptide based assay showed that patients with HTLV-I-associated myelopathy (HAM) or adult T cell leukemia (ATL) could be distinguished by their differential immune responsiveness to the immunodominant epitopes of the tax and rex proteins suggesting that such responses may relate to pathogenesis. Finally, an extended tax protein that appears to be a unique characteristic of most HTLV-IIb isolates was found to be useful for an immunoassay that could distinguish HTLV-IIa and HTLV-IIb infections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00352-12 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Chief, LMS, NIAID

Others:

K. Sturmhoefel, Ph.D., Visiting Fellow, LMS, NIAID

A. Chang, Ph.D., IRTA, LMS, NIAID

M. Halvorson, Ph.D., IRTA, LMS, NIAID

S. Otto, Ph.D., IRTA, LMS, NIAID

F. Martinon, Ph.D., Visiting Fellow, LMS, NIAID

W. Magner, Ph.D., IRTA, LMS, NIAID

COOPERATING UNITS *(if any)*

Laboratory of Immunology, NIAID (Dr. Ethan Shevach); Immunobiology Research Inst., (Dr. Scott Wadsworth)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Merosin is a 700 kDa multichain protein that has several properties in common with laminin, one of the major extracellular matrix proteins present in basement membranes. Both contain B1 and B2 light chains, but merosin has a distinct M-chain compared with the laminin A-chain. Merosin is present in the basement membrane of placenta, striated muscle, and peripheral nerve. Using the RT-PCR assay, we demonstrate that merosin, but not laminin, is also expressed in the thymus. (Previous reports of laminin expression in the adult thymus are probably in error). A partial cDNA sequence of the mouse merosin M-chain was 88% identical to the human M-chain, and the deduced amino acid sequences were 95% identical. 18-24% of adult mouse thymocytes bound to human merosin in vitro. Mg^{2+}, but not Ca^{2+}, was essential for binding. Binding was inhibited by antibodies recognizing VLA α_6 or β_1, suggesting that the VLA-6 integrin is a merosin receptor. An anti-β_4 integrin subunit mAb failed to inhibit binding, suggesting that the $\alpha_6\beta_4$ integrin was not involved. Thymocytes were fractionated into immature and mature populations based on their expression of the heat stable antigen, recognized by the J11d mAb. Virtually all thymocytes expressed VLA-6, but only immature thymocytes ($J11d^+$) bound to merosin. PMA treatment did not significantly increase the binding of $J11d^+$ thymocytes nor did it induce binding in the mature $J11d^-$ population. In contrast, both splenic T cells and unseparated lymph node cells showed enhanced binding to merosin after PMA stimulation. The expression of merosin in the thymus and its selective interaction with immature thymocytes suggest that thymocyte-merosin interactions may play a role in T cell development.

We have analyzed the expression and function of $\alpha4\beta1$ fibronectin binding integrin receptors on human thymocytes. A major portion of double positive ($CD4^+CD8^+$) human thymocytes express $\alpha4\beta1$ in a constitutively active form and adhere to fibronectin and vascular cell adhesion molecule 1. $\alpha4\beta1$ expression is similar on adherent and nonadherent populations, thus, activity reflects the receptor state and not simple expression. These adherent cells are immature, expressing high levels of CD4/CD8 and low levels of CD3 and CD69. In contrast, nonadherent cells possess the phenotype of thymocytes after positive selection, expressing intermediate levels of CD4 and/or CD8 and high levels of CD3 and CD69. The adherent population fails to respond to activation with anti-CD3 and fibronectin, whereas nonadherents exhibit an $\alpha5\beta1$-dependent proliferation. Differential regulation $\alpha4\beta1$ and $\alpha5\beta1$ receptors may provide a mechanism for controlling cellular traffic, differentiation, and positive selection of thymocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00522-07 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Sequence Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Chief, LMS, NIAID

COOPERATING UNITS *(if any)*

German Cancer Research Ctr. (G. Hammerling); UCLA (E. Sercarz), LI/NIAID (D. Margulies), Rosewell Park Memorial Inst., (B. Seon), LIG/NIAID (E. Long), University of Leiden (F. Koning), Univ. of Alabama (D. Pritchard), LMM/NIAID (T. Theodore), LIR/NIAID (J. Kehrl)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Biological Resources Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	0.1	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The LMS determined the amino acid sequences of about 650 samples. About 440 of these analyses were for unknown protein or peptides and the remainder were for quality control of synthetic peptides. Most of the unknown sequences determined (about 250) were of endogenous peptides eluted from mouse d and b haplotype class I molecules (LI/NIAID, D. Margulies) and from human HLA-A3, -B44, -B14, -B8 and -A1 molecules (LMS). From these analyses, amino acid residues in peptides requisite for peptide binding to particular class I molecules have been determined. This information has been used to search protein sequence databanks for potential antigenic T cell epitopes that are recognized by cytotoxic T lymphocytes which has important implications for vaccine development. Using such an approach several new antigenic epitopes recognized by cytotoxic T lymphocytes specific for influenza virus proteins and myelin basic protein have been identified. In addition, proteins and peptides from a large variety of other sources have been sequenced by the facility. These include CD22 binding proteins and other B cell specific proteins (Kehrl, LIR); HIV proteins (Theodore, LMM; Dayton, LIR); plasma sialoglycoprotein (spg-120) (Basta, LCI); female specific proteins (Coe, RML); constructs of malaria proteins; (Kaslow, LMR); mouse MHC class II endogenous peptides (Sercarz, UCLA); human MHC class II endogenous peptides (Hammerling, German Cancer Research Center; Koning, University of Leiden, the Netherlands; Lew, Walter and Eliza Hall, Australia) and group B Streptococcal hyaluronidase (Pritchard, Univ. Alabama).

A disulfide-linked heterodimeric antigen from human B leukemia cells was detected by radioimmunoprecipitation and Western blot analysis using a monoclonal antibody generated against a cell membrane antigen preparation from human B prolymphocytic leukemia cells and found to define an extracellular epitope of the smaller component (β chain) of a heterodimeric antigen on human B leukemia cells. The antigen from BALL-1, a human B leukemia cell line, and fresh (uncultured) B prolymphocytic leukemia cells was found to consist of a 44-49 kDa (α chain) and a 36-40 kDa (β chain) component. Determination of the amino terminal amino acid sequences of the α and β chains unequivocally identified them as the human mb-1 and B29 proteins, respectively. We detected three forms of the mb-1 protein which share an identical amino-terminal sequence and likewise, two forms of the B29 protein. A β-tubulin-like protein was found to co-purify with the mb-1-B29 antigen and we found that there is a strong amino acid sequence homology between the microtubule-binding domains of certain human microtubule-associated proteins and an intracellular segment of the human mb-1 protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00523-07 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Flow Cytometric Analyses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Chief, LMS, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Flow Cytometry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.25	.25	5.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Flow cytometry section has recently installed a FACScan for use by DIR investigators. This insturment is on indefinite loan from NCHGR and is linked to the FCS/NIAID network to allow for offsite data analysis. The FCS has purchased a new FACS Vantage multiparameter flow cytometer, which will probably arrive early FY '95. The FACStar Plus has been upgraded to perform simultaneous 5 color analysis. This upgrade is unique to this instrument and a similar insturment in Buffalo. This is new technology that is currently under beta testing at these facilities.

The amount of usage by DIR labs has increased 3539 hrs in FY 93 to 3659 hrs in FY 94. The percentage of this time devoted to sorting has increased from 48% to 56% of total usage. Therefore, for the first time, more than half of the usage time (including use of the user operated Epics Profile) was devoted to sorting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00543-07 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Recognition of Peptide Antigens by Virus-Specific Cytotoxic T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John E. Coligan, Ph.D., Chief, LMS, NIAID

Others:

M. DiBrino IRTA LMS/NIAID

M. Shields IRTA LMS/NIAID

COOPERATING UNITS *(if any)*

Neuroimmunology Branch, NINCDS (W. Biddison)

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.4	2.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The potential value of peptide binding motifs of HLA class I molecules for the prediction of viral epitopes presented to T cells has been analyzed for HLA-A1 and -A3. CTL generated against type A influenza virus recognize peptide epitopes derived from the nucleoprotein (NP) and basic polymerase 1 presented by HLA-A1, and epitopes derived from NP presented by HLA-A3. Distinct peptide binding motifs with characteristic anchor residues were previously identified for each of these class 1 molecules based on the sequences of endogenous peptides. Six peptides containing the HLA-A1 binding motif were identified within the sequences of the NP and basic polymerase 1 proteins, and one peptide containing the HLA-A3 motif was identified in the NP molecule. Two of the HLA-A1 binding peptides could sensitize target cells for lysis by influenza virus-immune CTL populations restricted by HLA-A1 and the one HLA-A3 NP peptide (NP 265-273 ILRGSVAHK) could sensitize target cells for lysis by HLA-A3 restricted influenza-immune CTL. Each peptide was also shown to be able to induce peptide-specific class I-restricted CTL in vitro, and the CTL generated against two of these peptides could specifically recognize virus-infected targets. Thus, peptide binding motifs can be used to construct immunogenic synthetic epitopes which are capable of inducing antiviral T cell-mediated immune responses.

Distinct amino acid residue motifs for peptides binding to HLA-A1 and HLA-B8 were identified by sequence analyses of reversed-phase HPLC fractions containing endogenous peptides derived from these HLA molecules. Fifteen different primary sequences were determined for HLA-A1 associated peptides. Common features among these peptide sequences were Tyr at the C-terminus, a negatively charged aa (usually Glu) at position 3 (P3), and pro at P4. Twenty-seven different primary sequence assignments were made for HLA-B8 associated peptides, most of which were eight aa in length. Lys, and in a few cases Arg, predominated at P3 and P5; Leu and Pro predominated at P2, and Leu was the preferred COOH-terminal residue. Eight of the HLA-A1 and HLA-B8 endogenous peptide sequences matched intracellularly expressed proteins found in protein sequence data bases. The HLA-A1 peptide-binding motif was then used to identify potential antigenic peptides from influenza A viral proteins that bound to HLA-A1 in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00595-04 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification Characterization and Function of Proteins of the Complement System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI: Carl H. Hammer, Senior Investigator, LMS/NIAID

Others:

Michael D. Knierman, Chemist, LMS/NIAID

Thomas L. Leto, Senior Staff Fellow, LHD/NIAID

COOPERATING UNITS *(if any)*

Michael M. Frank, Chairman, Dept. of Pediatrics, Duke Univ. Med. Ctr., Durham, NC 27710

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Biological Resource Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The full-length cDNA for a new plasma sialoglycoprotein (sgp120) was elucidated from two human fetal liver cDNA libraries by standard cloning and sequencing technologies. We have demonstrated that several forms of the gene that code for sgp120 exist due to spliced variants. The largest form expressed contains 893 amino acids including a 35 amino acid insert at residue 593. This information along with earlier biochemical and immunological data unequivocally confirms sgp120 as a new plasma protein. Sgp120 was first identified by its capacity to bind with the activated fourth component (C4b) of the classical complement pathway (CCP). Results to date support the idea that this form of the protein, sgp120-A, may regulate the activation of the CCP. Sgp120 is also cleaved by kallikrein of the intrinsic coagulation-kinin generating pathway to produce several protein fragments. Good pI agreement was obtained by computer prediction analysis of the translated amino acid sequence of the largest form containing the 35 amino acid splice region (pI = 6.54, 907 residues) and without (pI = 6.08, 872 residues) to that obtained by direct electrophoretic analysis of desialidated sgp120-A/I, pI = 6.3 and 5.8, respectively. This supports our hypothesis that the larger sgp120-A is a product of a spliced gene such as in clone D, the insert representing the C4b binding domain. Pigtryin (PT), a postulated analog of human inter-alpha-trypsin inhibitor (IATI) was shown to have a peptide match of 76% identity with 81% similarity to sgp120.

Thus, PT is most likely the pig analog of human sgp120 and not IATI. The mRNA of PT, identified as an acute phase reactant in a pig model of cardiogenic shock, is increased > 5 fold. Using an ELISA for sgp120 protein we have identified 2-3 fold increases in the serum of burn patients suggesting that sgp120 is also an acute phase reactant. Sgp120 as well shows strong homology to each of the three mature heavy chains of IATI. Homology to HC3 was most significant with 51% identity and 71% similarity over the 5' 619 amino acid residues upstream from the splice region.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00697-01 LMS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Characterization of Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter Sun, Ph.D., Sr. Staff Fellow, LMS, NIAID

Others:

Linda Hannick, Ph.D., Sr. Staff Fellow, LMS, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Structure

SECTION

Structural Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The setting up of the Structural Biology Section began with acquiring all of the necessary equipment for each of three areas of the section; X-ray diffraction, computer support and protein chemistry. The following describes the major equipment that has been acquired in the fiscal year of 94. X-ray diffraction unit consists of two Rigaku RU200 rotating anode X-ray generators as X-ray sources, two RAXIS-II imaging plate systems as 3-D diffraction data collection devices and an Enraf-Nonius precession camera for 2-D diffraction data collection. The computing support has four DEC Alpha 3000/800 series of workstations for speed computation, five Silicon Graphics Indigo 2-extreme workstations for computation and graphics interface, one Evans and Sutherland workstation as graphics interface two PC-486 computers and one MAC for simple graphics and word processing. The protein chemistry section has a 10L fermentor, a HPLC system and a FPLC system.

The second part of the process of structural biology establishment is to recruit individual researchers. Up to date, a Senior Research Fellow, Linda Hannick, has been recruited and a number of postdoctoral fellows are in the process to be recruited to the section.

The third component is to initiate research projects. Projects currently in progress are i) crystallization and structure determination of TGF-β Receptor, TGF-β1 LAP and their Complexes; ii) crystallographic studies of cytokines, like CTGF (a connective tissue growth factor) and IL-13; iii) expression, purification and crystallization of immune related receptors, including type 1 interferon receptor and TCR; iv) structure determination of a bioluminescent protein aequorin.

Laboratory of Cellular and Molecular Immunology
1994 Annual Report
Table of Contents

Z01-AI Project Number		Page
	Summary	7-1
00485-08	T Cell Activation	7-6
00613-04	T Cell Subsets	7-7
00486-08	T Cell Differentiation	7-8
00716-01	Molecular Mechanisms of Programmed Cell Death	7-9
00581-05	T Cell Memory and Tolerance	7-10

PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Cellular and Molecular Immunology
October 1, 1993 through September 30, 1994

Introduction

The Laboratory of Cellular and Molecular Immunology was created in November, 1986. The major task is to perform research on thymus-derived (T) lymphocytes. Its objective is to understand how these critical basic cells of the immune system differentiate and function. The approach is to define problems in whole animal systems, set up *in vitro* analogs in tissue culture, and determine the molecular basis for the phenomena. The Laboratory employs techniques from the disciplines of cellular immunology, molecular biology, and protein biochemistry.

During the 8th year, the Laboratory has pursued five major areas of research: the development of $\gamma\delta$ T cells, positive selection of $\alpha\beta$ T cells, negative selection of $\alpha\beta$ T cells, and new models for T cell memory and T cell tolerance.

T Cell Subsets

The development and function of the minor subset of $CD4^-8^-$ double negative (DN) TCR$\alpha\beta$ T cells and their relationship to the mainstream $CD4^+$ and $CD8^+$ T cells have been the subject of much intense research. We described recently a subset of mature $CD4^+$ mouse thymocytes that is very similar to the DN T cells. It shares the membrane expression of the activation markers CD44 and Ly6C and the natural killer cell marker NK1.1. This population also shares the unique potential to immediately secrete a large set of lymphokines typical of Th0-activated T cells such as interleukin-4 (IL-4), IL-5, and interferon γ (IFN-γ). Thus, its physiologic role may be to influence the Th1/Th2 differentiation of an immune response early after the introduction of antigen. In the past year, we have focused on the development of this T cell subset and characterized its T cell receptor repertoire. The selection of these cells in the thymus requires MHC class I expression and not MHC class II, CD4, or CD8 molecules. The selecting element appears to be a nonclassical MHC class Ib molecule expressed on bone marrow-derived cells but not on thymic stromal cells. The selected T cells express a very defined set of receptors consisting of a single V α chain, Vα14 J281, and three V βs: Vβ8, Vβ7, and Vβ2. A similar receptor family was found in human double negative T cells. These results suggest that this special subset is uniquely selected to recognize a narrow set of evolutionarily conserved ligands.

[A. BENDELAC, D. PHAM, L. CHIODETTI, J. SALO, O. LANTZ, AND R. H. SCHWARTZ]

T Cell Activation

In order to understand the development of the thymus both in terms of T cell differentiation and the stromal cell environment, we have undertaken a molecular approach to identify the genes expressed in this tissue. Our strategy is to make two cDNA libraries: one from total thymus of 129 mice, the other from BALB/c mice expressing the SCID mutation. The latter has a developmental arrest at the IL-2 receptor α high double negative stage of T cell development; thus, the cDNA library from this mouse will be enriched for genes expressed in early T cell precursors and the stromal cells. We are currently developing several methods for screening these libraries, including subtractive hybridization, massive cDNA sequencing, probing with PCR-based libraries generated from rigorously defined T cell subsets, and differential display. At this early stage of the project, we have prepared the two cDNA libraries from total and SCID thymus. Although we have only just begun some preliminary sequencing, we have already identified the mouse ARF-3 protein (a small GTP binding protein involved in vesicular transport) and a new β3 integrin (involved in cell adhesion), as well as several genes with no known homology to anything in the GenBank data base.

[M. Kim, S. Umlauf, S. Kitagawa, M. Manoussakis, and R. H. Schwartz]

T Cell Differentiation

Mature T cells develop in the thymus through a series of differentiation events that are either genetically programmed or dependent on external signals provided in the microenvironment. Some of these signals induce death or maturation, promote proliferative phases, or are responsible for the divergence of lineages. Our major efforts focus on γδ/αβ lineage commitment, the requirements for γδ development, selective events in the αβ lineage, and the mechanism of the CD4/CD8 lineage decision. This year, studies on αβ T cells showed that—for some T cells—more than one MHC may be used to drive the cell to full maturation, supporting the notion that positive selection is a multistep event. Somewhat surprisingly, the elimination of CD4 in mice bearing a transgenic class II-dependent TCR altered the lineage decision such that significantly more cells matured as CD8 T cells. These data, taken together with other studies showing that constitutive expression of a CD8 transgene is very inefficient at promoting the maturation of $CD4^+$ cells with class I MHC-specific TCR, argue against a model in which lineage decisions are made irrespective of coreceptor usage. Investigations of γδ T cell development indicated that the type of MHC allele can determine the death or survival and functional capacity of mature cells. The maturation of some γδ T cells appears to require MHC displayed on hematopoietic cells rather than on thymic epithelium. In TCRαβ transgenic mice, a population of $CD4^-8^-$ T cells is generated bearing the transgenic TCR with phenotypic and functional properties of γδ T cells, suggesting that γδ/αβ lineage commitment may occur independent of TCR gene rearrangement. Both γδ and αβ antigen receptors exist on some cells in the epidermis, while the γδ TCR is not observed at all in the thymus, the reproductive epithelium, or other lymphoid organs. These results indicate that the suppression of endogenous γδ TCR by the expression of transgenic TCRαβ is related to the timing of TCR gene rearrangement and/or expression.

[E. Schweighoffer, K. Terrence, and B. J. Fowlkes]

Molecular Mechanisms of Programmed Cell Death

A number of studies have shown that T cell negative selection is mediated by programmed cell death (apoptosis) and can be blocked by inhibitors of macromolecular synthesis, indicating that mRNA(s) with a short half-life encoding a protein(s) with high turnover rate and/or that synthesis of new mRNA(s) and its translation into protein(s) is required. The major objective is the dissection of the intracellular events linking the TCR/Ag+MHC interaction to cell death. Specifically, the project will progress in two distinct but connected stages: *1)* isolation and identification of the genes involved in negative selection, and *2)* characterization of the biochemical processes responsible for their functional activation. The strategic approach is based on the hypothesis that programmed cell death could be blocked by specifically inhibiting translation of required mRNAs. This goal can be achieved by expressing in the cells adequate levels of specific antisense RNAs. Since this hypothesis cannot be tested *in vivo*, we have used a mouse T cell hybridoma (3DO) which undergoes programmed cell death upon TCR triggering. 3DO's apoptosis, as in negative selection of normal T cells, requires ongoing mRNA transcription and protein synthesis. Given the similarities between TCR-induced 3DO cell death and negative selection, this hybridoma can be used as an *in vitro* model for the initial identification and characterization of genes involved in the deletion of self-reactive T cells. To this end, we have constructed cDNA libraries into eukaryotic expression vectors using an mRNA source enriched in apoptotic genes. Upon transfection of the libraries, 3DO has been triggered for apoptosis. If our assumption is correct, cells transfected with plasmids encoding cDNAs necessary to carry out the death process and expressing antisense RNAs should be rescued from TCR-induced death. Therefore, the plasmids recovered from surviving cells should contain "apoptotic genes." Once defined, the requirements for the functional activation of these molecules will be studied in more detail.

[V. PASQUALE, E. LACANA, AND L. D'ADAMIO]

T Cell Tolerance and Memory

This year, Dr. Matzinger published two new theoretical ideas, one in the area of T cell memory, the other in the area of T cell tolerance. The former divides memory into two discrete mechanisms. The first is an antigen-capture mechanism by follicular dendritic cells that allows presentation to activated T cells over a several-year period; she refers to this as short-term memory. The second, or long-term, memory involves cross-reactions with environmental antigens that allows the T cells to remain in the body for unlimited periods of time. These ideas were published in a *Nature* News & Views in June, 1994. The tolerance model is far more extensive and is difficult to summarize in a few sentences. It represents a radical departure from the current dogma with its emphasis on self-nonself discrimination. Instead, Dr. Matzinger places the emphasis on protection from danger. In this model, death from apoptosis is ignored by the immune system. Death during inflammation, however, releases signals that activate local antigen-presenting cells (APCs). These capture antigens from their environment, including any viral or bacterial antigens, take them to the nearest lymph node, and present them to passing T cells. Without the signals released from cells dying abnormal deaths, there is no activation of APCs and no immune response. To deal with self tolerance, the theory begins by acknowledging that T cells cannot become tolerant in

the thymus of all the antigens presented by the other organs of the body. All that is necessary is tolerance to APCs, and this is easily accomplished with the rule that thymocytes pass through a stage in which their response to antigen is suicide. Thus, any T cell specific for APCs (which are normally found in the thymus) will be deleted. To ensure that T cells specific for other peripheral body tissues are inactivated, the theory borrows a line from earlier theories of T cell activation and sets the rule that T cells require two signals in order to be activated: one arising from antigen recognition, and the other coming from the APC. Any T cell that recognizes an antigen presented by any non-APC tissue is deleted by the lack of a second signal. Thus, normal tissues have three roles. They perform their normal bodily function (*e.g.*, kidneys filter plasma and the heart pumps blood), they alert the immune system of a dangerous pathogen, and they induce tolerance to themselves. This view of the immune system explains many phenomena that the old self/non-self view does not and it offers several new strategies for the treatment of tumors and for successful organ transplantation.

[L. BONNEY, O. LANTZ, L. YUAN, F. DIROSA, M. EPSTEIN, AND P. MATZINGER]

Administrative, Organizational, and Other Changes

During the past year, LCMI officially set up two new Sections: the Thymocyte Differentiation Section, headed by Dr. B. J. Fowlkes, and the T Cell Tolerance and Memory Section, headed by Dr. Polly Matzinger. In addition, we recruited Dr. Luciano D'Adamio to head the T Cell Molecular Biology Unit in a tenure-track position. He has brought two new postdoctoral Fellows, Drs. Vito Pasquale and Emanuela Lacana, as well as one new technician, Ms Brenda Lawrence. The T Cell Activation Section has added four new Fellows: Drs. Moon Kim, Jonathan Sado, Jack Ragheb, and Yongrui Zou. Two Fellows have departed, Drs. Scott Umlauf and Albert Bendelac. In the T Cell Tolerance and Memory Section, Drs. Francesca diRosa and Michelle Epstein have arrived, while Dr. Olivier Lantz has departed. Finally, the T Cell Differentiation Section is sponsoring one Howard Hughes Scholar, Ms Kathleen Terrence.

Honors, Awards, and Scientific Recognition

Dr. Schwartz is a member of the editorial boards of *Science, International Immunology, Current Opinion in Immunology*, and *Immunology Today*. This year, he was chosen to be on the editorial board of a new journal, *Immunity*, published by Cell Press. During the past year, he was the invited outside speaker at the annual meeting of the Australian Society of Immunology, Victoria Group, held at Buffalo Mountain, Victoria, Australia. Afterward, he presented the prestigious Colin Syme Lecture at the Walter and Eliza Hall Institute in Melbourne, Australia; this was followed by a lecture to the Sydney Group of the Australian Society of Immunology in Sydney, Australia. In January, he was a symposium speaker at the *1994 Keystone Symposium on Molecular Mechanisms Common to Types I and II Diabetes* held in Park City, Utah. In February, he taught in the Winter Advanced Course for Immunology and Infectious Diseases, 1994, at Furusato Village in Chiba, Japan, and spoke at a meeting on *T Cell Selection and Tolerance* held in Tampa, Florida. In April, he chaired a workshop on co-stimulation at the *Keystone Symposium on Lymphocyte Activation* held in Keystone, Colorado. In May, he spoke at the NCI *Workshop on Apoptosis in Immunology*

and Hematopoiesis in Chevy Chase, Maryland. In July, he gave a lecture on tolerance in the American Association of Immunologists' Course in Advanced Immunology at Montana State University in Boseman, Montana. In August, he spoke at a meeting on *T Cell Signaling* held at the Ontario Cancer Institute in Toronto, Ontario, Canada. Finally, in September, he co-organized the *International Conference on Programmed Cell Death* held on the NIH campus and sponsored by the Fogarty International Center and the Cell Growth and Growth Hormone Forum. This meeting had 21 speakers and an attendance of 160.

Dr. B. J. Fowlkes received the American Association of Immunology Junior Investigator Award for outstanding contributions to the field of Immunology. She serves on the editorial boards of *The Journal of Experimental Medicine, The Journal of Biomedical Science*, and *The Journal of Immunology*. She serves on the Committee on the Status of Women for the American Association of Immunology and as the Women Scientist Advisor to the Scientific Director of NIAID. During the past year, Dr. Fowlkes was invited to chair the *Lymphocyte Differentiation and Lineage Commitment Workshop* at the NIH Research Festival and the T Cell Development Workshop at the *Keystone Symposium on Lymphocyte Activation* in Keystone, Colorado. She has also been invited to organize the *Keystone Symposium on Lymphocyte Activation* to be held in Keystone, Colorado, in the spring of 1996. Dr. Fowlkes was an invited speaker at the WHO/HHS/IASIA meeting on *Immunology and Aging: Vaccine Development for Immunization of the Elderly* held at NIH and the Fifty-seventh Annual Meeting of the Peripatetic Club at the Howard Hughes Medical Institute in Bethesda, Maryland. Dr. Fowlkes was invited to give research or teaching seminars to the Department of Immunology and Molecular and Medical Genetics at the University of Toronto (Toronto, Canada), at the FAES Graduate School at NIH, and the Experimental Immunology Branch, National Cancer Institute, NIH.

Dr. L. D'Adamio was a speaker at the meeting on *Fundamental and Experimental Aspects of Autoimmunity* held in Acquafredda di Maratea in May 1993.

Dr. Matzinger is a member of the editorial boards of *Immunobiology, Seminars in Immunology, International Archives of Allergy and Immunology and Current Opinion in Immunology*. She is also a regular contributer to "*Real Science*" a monthly letter for high school students. During the past year she was featured in a Documentary film about Cell Signals and Cell Death, commissioned for the European television station, ARTE.
She was the keynote speaker at the *Genetics Institute's Yearly research retreat*, Boston, MA; invited symposium speaker at the *Canadian Society for Immunology*, Montreal, Canada; invited symposium speaker at the *American Society of Transplant Surgeons Symposium*, Chigago; invited symposium speaker at the *IUIS meeting on Autoimmunity*, Strasbourg, France; invited symposium speaker at the *British Society of Immulogy Conference* on Ontogeny and Tolerance, TATRA, Slovakia and workshop chairperson at Keystone Symposium on T lymphocytes.

Dr. Matzinger was invited to present research seminars on her major new theory of the immune system at the National Institutes of Health, Bethesda MD; the Fred Hutchison Cancer Center, Seattle Washington; the John's Hopkins University, Baltimore, MD; the Wistar Institute, Philadelphia, PA; Anergen, San Francisco, CA; and Hoffman La Roche, Nutley, NJ.

Dr. Matzinger has been asked to teach in two international courses: The Third Central European Summer School of Immunology, Tatra, Slovakia ASI?FIMSA graduate course in Immunology, Brisbane, Australia. She also taught Immulogy at the University of MD, College Park and lectured on the Immune system at the NIH course for grade school children as well as Introduction to Biomedical Research, NIH.

Dr. Matzinger is a consultant for the Genetics Institute, Boston, MA and Marion Merrel Dow, Kansas City, MO.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00485-08 LCMI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

T Cell Activation

PRINCIPAL INVESTIGATOR	Ronald H. Schwartz	Chief	LCMI, NIAID
Others:	Moon Kim	Visiting Fellow	LCMI, NIAID
	Scott Umlauf	Staff Fellow	LCMI, NIAID
	Satoru Kitagawa	Visiting Fellow	LCMI, NIAID
	Menelaus Manoussakis	Visiting Fellow	LCMI, NIAID

COOPERATING UNITS *(if any)*

Lymphocyte Biology Section, Laboratory of Immunology, NIAID, NIH

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	4.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

In order to understand the development of the thymus, both in terms of T cell differentiation and the stromal cell environment, we have undertaken a molecular approach to identify the genes expressed in this tissue. Our strategy is to make two cDNA libraries: one from total thymus of 129 mice, the other from BALB/c mice expressing the SCID mutation. The latter has a developmental arrest at the IL-2 receptor α high double negative stage of T cell development; thus, the cDNA library from this mouse will be enriched for genes expressed in early T cell precursors and the stromal cells. We are currently developing several methods to screen these libraries, including subtractive hybridization, massive cDNA sequencing, probing with PCR-based libraries generated from rigorously defined T cell subsets, and differential display.

At this early stage of the project, we have prepared the two cDNA libraries from total and SCID thymus. Although we have only just begun some preliminary sequencing, we have already identified the mouse ARF-3 protein (a small GTP binding protein involved in vesicular transport) and a new β3 integrin (involved in cell adhesion), as well as a third gene with no known homology to anything in the GenBank data base.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00613-04 LCMI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

T Cell Subsets

PRINCIPAL INVESTIGATOR	Ronald H. Schwartz	Chief	LCMI, NIAID
Others:	Albert Bendelac	Visiting Associate	LCMI, NIAID
	David Pham	IRTA Fellow	LCMI, NIAID
	Lynda Chiodetti	Biologist	LCMI, NIAID
	Jonathan Salo	Commissioned Officer	LCMI, NIAID
	Olivier Lantz	Visiting Fellow	LCMI, NIAID

COOPERATING UNITS *(if any)*

Department of Microbiology & Immunology, Howard Hughes Institute, U. Cal-San Francisco; Flow Cytometry Section, Biological Resources Branch, NIAID; Animal Care Branch, NIAID.

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Activation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.25	4.25	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

The development and function of the minor subset of $CD4^-8^-$ double negative (DN) TCRαβ T cells and their relationship to the mainstream $CD4^+$ and $CD8^+$ T cells have been the subject of much intense research. We described recently a subset of mature $CD4^+$ mouse thymocytes that is very similar to the DN T cells. It shares the membrane expression of the activation markers CD44 and Ly6C and the natural killer cell marker NK1.1. This population also shares the unique potential to immediately secrete a large set of lymphokines typical of Th0-activated T cells such as interleukin-4 (IL-4), IL-5, and interferon γ (IFN-γ). Thus, its physiologic role may be to influence the Th1/Th2 differentiation of an immune response early after the introduction of antigen. In the past year, we have focused on the development of this T cell subset and characterized its T cell receptor repertoire. The selection of these cells in the thymus requires MHC class I expression and not MHC class II, CD4, or CD8 molecules. The selecting element appears to be a nonclassical MHC class Ib molecule expressed on bone marrow-derived cells but not on thymic stromal cells. The selected T cells express a very defined set of receptors consisting of a single V α chain, Vα14 J281, and three V βs: Vβ8, Vβ7, and Vβ2. A similar receptor family was found in human double negative T cells. These results suggest that this special subset is uniquely selected to recognize a narrow set of evolutionarily conserved ligands.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00486-08 LCMI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

T Cell Differentiation

PRINCIPAL INVESTIGATOR	B. J. Fowlkes	Senior Investigator and Section Chief	LCMI, NIAID
Others:	E. Schweighoffer	Visiting Fellow	LCMI, NIAID
	K. Terrence	Howard Hughes Medical Scholar	LCMI, NIAID
	E. O'Connell	Technician	LCMI, NIAID
	H. Dinh	Stay-in-school	LCMI, NIAID

COOPERATING UNITS *(If any)* Flow Cytometry Section, BRB, NIAID (R. Swofford, C. Eigsti); Animal Care Branch, DIR, NIAID (P. Golway); Department of Molecular and Cell Biology, University of California, Berkeley (E. Robey); Laboratory of Immunology, NIAID (J. van Meerwijk, R. Germain); Section on Pulmonary and Molecular Immunology, NHLBI (W. Leonard); Howard Hughes Medical Institute, Children's Hospital, Boston (F. Alt).

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

Thymocyte Differentiation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	3.0	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

Mature T cells develop in the thymus through a series of differentiation events that are either genetically programmed or dependent on external signals provided in the microenvironment. Some of these signals induce death or maturation, promote proliferative phases, or are responsible for the divergence of lineages. Our major efforts focus on γδ/αβ lineage commitment, the requirements for γδ development, selective events in the αβ lineage, and the mechanism of the CD4/CD8 lineage decision.

Our studies in αβ T cells show that—for some T cells—more than one MHC may be used to drive the cell to full maturation, supporting the notion that positive selection is a multistep event. Somewhat surprisingly, the elimination of CD4 in mice bearing a transgenic class II-dependent TCR altered the lineage decision such that significantly more cells matured as CD8 T cells. These data, taken together with other studies showing that constitutive expression of CD8 transgene is very inefficient at promoting the maturation of $CD4^+$ cells with class I MHC-specific TCR, argue against a model in which lineage decisions are made irrespective of coreceptor usage.

Investigations of γδ T cell development indicate that the type of MHC allele can determine the death or survival and functional capacity of mature cells. The maturation of some γδ T cells appears to require MHC displayed on hematopoietic cells rather than on thymic epithelium. A discrepancy exists in that mature γδ cells are present in lymph nodes and not the thymus of β2m-deficient mice. These studies are a necessary prerequisite to identifying the selecting ligand for γδ T cells, if it exists.

In TCR αβ transgenic mice, a population of $CD4^-8^-$ T cells is generated bearing the transgenic TCR with phenotypic and functional properties of γδ T cells, suggesting that γδ/αβ lineage commitment may occur independent of TCR gene rearrangement. Both γδ and αβ antigen receptors exist on some cells in the epidermis, while γδ TCR is not observed at all in the thymus, the reproductive epithelium or other lymphoid organs. These results indicate that the suppression of endogenous γδ TCR by the expression of transgenic TCRαβ is related to the timing of TCR gene rearrangement and/or expression. Some γ rearrangements occur in both $CD4^-8^-$ and $CD4^+8^-$ subpopulations of TCRαβ transgenic mice; whether these are quantitatively different or differ in the frequency of productive rearrangement is under investigation. The latter results should bear on the nature of lineage commitment and allelic exclusion in γδ T cells.

PHS 6040 (Rev 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00581-05 LCMI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

T Cell Tolerance and Memory

PRINCIPAL INVESTIGATOR

	Polly Matzinger	LCMI, NIAID	Chief,
Others:	Elizabeth Bonney	Medical Staff Fellow	LCMI, NIAID
	Lin Yuan I	Visiting Fellow	LCMI, NIAID
	Olivier Lantz	Visiting Fellow	LCMI, NIAID
	Michelle Epstein	Visiting Associate	LCMI, NIAID
	John Ridge	GS-11 Technician	LCMI, NIAID
	Rosemary Hunziker	Senior Staff Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

T Cell Tolerance Section, Laboratory of Cellular and Molecular Immunology, NIAID, NIH

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Tolerance Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL WOMAN/MAN-YEARS:	PROFESSIONAL:	OTHER:
3/3	5.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

CONTINUED FROM LAST YEAR: TOLERANCE: 1)Neonatal tolerance Female mice can be primed by male dendritic cells or tolerized by male spleen cells up to day 24 after birth. Thus neonates are not especially tolerizable. They can be immunized by professional APCs as long as the inoclum does not contain a large proportion of non-APC cells. 2) Maternal tolerance Using quantitative PCR for the Y chromosome we occasionally find male cells in the organs of pregnant mice. This traffic is very sporadic however and we conclude that it is unlikely to be a major mechanism by which the fetus alters the mother's immune response. 3) Results of tolerance in Tg mice To test which haplotypes support the development of a Tg TCR derived from an F1 mouse, we created T cell clones from F1 mice, isolated the genes for their antigen specific receptors and generated Tg mice. The first two transgenics are now being bred to create the appropriate test animals. Two more are on the way.

THE THYMIC STEM CELL: is it precommitted? Frequency estimates indicate that each 11 day fetal thymus lobe contains a single CFUS stem cell . Thus there are uncommitted stem cells in the early fetal thymus.

T CELL MEMORY: We are studying killer cell memory in mice depleted of T helper cells. Preliminary results suggest that killer cell memory lasts at least 3 months in the absence of T helper cells.

NEW THIS YEAR Two major theories, one on memory and one on the fundamental "raison d'etre" of the immune system.

On Memory: Both short and long term memory are maintained by re-stimulation of memory cells: short term memory by antigen retained by Follicular Dendritic cells and long term memory by cross-reactive environmental antigens or by re- infection with the original pathogen.

The immune system recognizes "danger" not "non-self": "Danger" is defined by the immune system as cell stress or non-apoptotic death, eg. lysis caused by a cut or a viral infection. This activates local antigen presenting cells (APCs) which take captured antigens to the nearest lymph node and present them to passing T cells. Immune responses are only initiated when abnormal death activates APCs. T cells able to see the normal surface MAP of APCs are deleted in the thymus and any T cells able to see other bodily tissues are deleted in the periphery. This peripheral deletion occurs because resting T cells are programmed to die if they bind antigen unless they receive a special APC-derived activating signals. Thus any T cell specific for a peripheral (non-APC) tissue dies when it binds to that tissue. This view of the immune system explains many phenomena that the old self-non-self view does not and it offers several new strategies for the treatment of tumors and for succcessful organ transplantation .

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00716-01 LCMI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

Molecular Mechanisms of Programmed Cell Death

PRINCIPAL INVESTIGATOR	Luciano D'Adamio	Visiting Scientist and Section Chief	LCMI, NIAID
Others:	Pasquale Vito	Fogarty Visiting Fellow	LCMI, NIAID
	Emanuela Lacana	Fogarty Visiting Fellow	LCMI, NIAID
	Brenda Lawrence	GS-7 Technician	LCMI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Molecular Immunology

SECTION

T Cell Molecular Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

T cells recognize peptides bound to MHC molecules using a clonotypic receptor (TCR). Depending on the differentiation state of the T lymphocyte (*i.e.*, responsiveness to a second signal) and/or the context in which the antigenic peptide is presented (*i.e.*, availability of a second signal), this interaction can initiate distinct molecular programs leading to opposite outcomes (T cell activation, unresponsiveness, or death). A precise regulation of these responses results in a system that is both immunocompetent (able to be activated by and eliminate dangerous microorganisms) and self tolerant (able to eliminate or anergize T cell populations bearing TCRs specific for self antigens). The main process through which immunotolerance is achieved involves elimination of potentially dangerous T cells (negative selection or clonal deletion). A number of studies have shown that negative selection is mediated by programmed cell death (apoptosis) and can be blocked by inhibitors of macromolecular synthesis, indicating that mRNA(s) with a short half-life encoding a protein(s) with high turnover rate and/or that synthesis of new mRNA(s) and its translation into protein(s) is required. Our major objective is the dissection of the intracellular events linking the TCR/Ag+MHC interaction to cell death. Specifically, the project will progress in two distinct but connected stages: *1)* isolation and identification of the genes involved in negative selection and *2)* characterization of the biochemical processes responsible for their functional activation. Our strategic approach is based on the hypothesis that programmed cell death could be blocked by specifically inhibiting translation of required mRNAs. This goal can be achieved by expressing in the cells adequate levels of specific antisense RNAs. Since this hypothesis cannot be tested *in vivo*, we have used a mouse T cell hybridoma (3DO) which undergoes programmed cell death upon TCR triggering. 3DO's apoptosis, as in negative selection of normal T cells, requires ongoing mRNA transcription and protein synthesis. Given the similarities between TCR-induced 3DO cell death and negative selection, this hybridoma can be used as an *in vitro* model for the initial identification and characterization of genes involved in the deletion of self-reactive T cells. To this end, we have constructed cDNA libraries into eukaryotic expression vectors using an mRNA source enriched in apoptotic genes. Upon transfection of the libraries, 3DO has been triggered for apoptosis. If our assumpion is correct, cells transfected with plasmids encoding cDNAs necessary to carry out the death process and expressing antisense RNAs should be rescued from TCR-induced death. Therefore, the plasmids recovered from surviving cells should contain "apoptotic genes." Once defined, the requirements for the functional activation of these molecules will be studied in more detail. The physiologic relevance of any finding must be subsequently confirmed in an *in vivo* animal model.

PHS 6040 (Rev 5/92)

LABORATORY OF CLINICAL INVESTIGATION

1994 Annual Report

Table of Contents

ZO1 AI Project Number		Page
Lab Chief's Summary		5-1
0057-21	Basic Studies of Pathogens Causing Cryptococcosis--Kwon Chung	5-12
0058-20	Pathogenesis and Chemotherapy of Herpesvirus Infection in Man--Straus	5-13
00249-13	The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders--Metcalfe	5-14
00354-12	Immunoregulatory Defects in Inflammatory Bowel Disease--Strober	5-15
00356-12	Studies of the Regulation of IgA Immunoglobulin Synthesis--Strober	5-16
00430-10	Molecular Biology of Varicella Zoster Virus Infection--Cohen	5-17
00432-10	Regulation of Immune Responses in Humans and Non-Human Primates--Strober	5-18
00470-09	Chronic Epstein Barr Virus Infection and Chronic Fatigue Syndrome--Straus	5-19
00513-07	Molecular Biology of Mast Cell Growth and Differentiation--Metcalfe	5-20
00548-06	Prevention of Genital Herpes Simplex Infection--Straus	5-21
00589-05	Identification of Complement Resistance Determinants in Escherichia coli--Russo (Terminated)	5-22
00590-05	Studies of Primary Immunodeficiency Diseases--Strober	5-23
00607-04	Regulation of Cytokine Gene Expression in Mast Cells--Metcalfe	5-24

Table of Contents (Cont.)

00617-04	Regulation of Capsule Synthesis in a Pathogenic Escherichia coli Isolate--Russo (Terminated)	5-26
00621-04	Molecular Biology of Transformation by Epstein-Barr Virus--Cohen	5-25
00650-03	Treatment of Chronic Hepatitis B Virus Infection--Straus	5-27
00651-03	Mechanism of Recurrent Urinary Tract Infections in Women--Russo (Terminated)	5-28
00654-03	Molecular Genetics, Cell Biology and Biochemistry of Candida albicans Maltase--Bennett	5-29
00655-03	Studies of Pathogenic Fungi--Bennett	5-30
00657-03	Basic Studies of Candida Species--Kwon Chung	5-31
00674-02	Molecular Genetics of Varicella-Zoster Virus--Cohen	5-32
00680-02	Novel Cytokines from Activated Macrophages--Farber	5-33
00687-02	Structure-Function Analysis of the Immediate-Early Protein IE62 of Varicella-Zoster Virus--Perera	5-34
00688-02	Analysis of Herpesvirus Interaction with Cell Surface Molecules--Williams	5-35
00704-01	Regulation of Cytokine Production in Animals Infected with Histoplasma Capsulatum--Seder	5-36
00705-01	Regulation of Cytokine Production from patients Infected with HIV and Tb--Seder	5-37
00708-01	Molecular and Clinical Basis of Food Allergy--Metcalfe	5-38
00709-01	Lymphokine Profiles in Asthma and Allergic Diseases--Metcalfe	5-39
00710-01	Cellular Homologs of Herpesvirus Genes--Cohen	5-40

Summary Report
Laboratory of Clinical Investigation
October 1, 1993 to September 30, 1994

Stephen E. Straus M.D.
Chief, Laboratory of Clinical Investigation
NIAID

Introduction

The Laboratory of Clinical Investigation (LCI) differs in several regards from all of the other intramural Laboratories within NIAID. Its interests span a wide range of both clinical and basic research problems pertaining to allergic, immunologic, and infectious diseases. In pursuing its mission, the LCI has changed and evolved steadily through the years with the recruitment and departure of staff and in keeping with fundamental research advances and opportunities.

In February, 1991, the LCI's course was re-directed when Dr. Stephen Straus was appointed Laboratory Chief, replacing Dr. Michael M. Frank in that position. This is the first major outside review of the LCI since that appointment. LCI senior staff view this review as a pivotal assessment of the resources currently allocated to the LCI and of its new research direction and recent accomplishments.

History

The LCI was formed in 1957 to provide a clinical arm to the NIAID Intramural Program. From that time until 1991, the LCI Chief and the NIAID Intramural Clinical Director were one and the same person. Under the direction of Dr. Vernon Knight, a noted influenza virologist and still Professor of Microbiology and Immunology at Baylor University, the LCI comprised largely virologists who conducted basic and human volunteer studies of respiratory and gastrointestinal viruses and vaccines. Dr. Gordon Douglas is one of the LCI's illustrious alumni from the Knight era.

In 1968, Dr. Sheldon Wolff assumed the position of LCI Chief and NIAID Clinical Director. At that point, the LCI occupied the 11 East and West nursing units fully, a small outpatient clinic on the first floor of the Clinical Center, and about two-thirds of the laboratory space on the 11th floor (Figure 1). Dr. Wolff's philosophy was to establish in the LCI a large number of small units and sections build around promising young allergists, immunologists, and infectious disease physicians who would concentrate on problems relating to inflammatory response and host defenses. His own research led to the initiation of enormously successful studies of fever, Wegener's granulomatosis, and vasculitis, among others. Some of these programs were taken with Dr. Wolff when he left in 1977 to assume the position of Chairman of Medicine of Tufts

University-New England Medical Center, a position that he occupied until his recent, unfortunate death. Young scientists who emerged from the LCI as Fellows or staff during Dr. Wolff's tenure here include Drs. Richard Root, Charles Dinarello, Anthony Fauci, John Gallin, Ethan Shevach, Raphael Dolin, Alan Kaplan, Dean Metcalfe, John Bennett, and Michael Frank who was appointed LCI Chief and Clinical Director in 1978.

Dr. Frank, a noted pediatric immunologist, expanded his own program of research in complement-mediated disorders and consolidated LCI space into a smaller number of larger research groups. Some of Dr. Wolff's other senior staff had departed, and remaining Sections were headed by Drs. Frank, Anthony Fauci, John Gallin, Michael Kaliner, and John Bennett. With the closing of the NIAID Laboratory of Microbiology in the late 1970s, Dr. Kwon-Chung, an accomplished medical mycologist, was moved to the 11th floor to join Dr. Bennett's Section. Dr. Frank recruited Dr. Dean Metcalfe back from Harvard to head an additional allergy group concentrating on mast cell disorders, and he recruited Dr. Stephen Straus from Washington University to replace Dr. Dolin as Head of the Medical Virology Section.

In the early 1980s, most of the LCI space within the 11th floor B corridor was turned over to Dr. Anthony Fauci upon the establishment of his own, new Laboratory of Immunoregulation, but additional space was given the LCI as the new Ambulatory Care Research Facility (ACRF) was added to the front of the NIH Clinical Center (Figure 2). This addition also permitted a meaningful expansion of LCI's outpatient research protocols.

In the mid-1980s, Dr. Warren Strober was recruited from the Metabolism Branch, NCI, to create a new Section in the LCI to study problems in mucosal immunity, immunoglobulin disorders, and inflammatory bowel diseases. In the late 1980s, Dr. Frank recruited Dr. Harry Malech from Yale to join Dr. John Gallin in the Bacterial Diseases Section, and he asked Dr. Karen Lomax, a postdoctoral Fellow with Dr. Malech, to form a new Gene Therapy Unit in the LCI. In 1989, Dr. Frank recruited Dr. Thomas Russo from Tufts University to establish a new program in bacterial pathogenesis as part of his own Section.

Through the years of Dr. Frank's direction, notable Fellows and staff emanating from the LCI included Drs. Barton Haynes, H. Clifford Lane, Robert Schooley, Eric Brown, Keith Joiner, and Thomas Casale.

At the time of the last LCI Board of Scientific Counselors' review in May, 1990, the organizational structure and senior staffing of the Laboratory was as follows:

Laboratory Chief & Clinical Director	Michael Frank, M.D.
Clinical Immunology Section	Michael Frank, M.D., Section Head Carl Hammer, Ph.D., Tenured Scientist Thomas Russo, M.D., Tenure-Track Scientist

Allergic Diseases Section	Michael Kaliner, M.D., Section Head Robert Hohman, Ph.D., Tenure-Track Scientist Martha White, M.D., Tenure-Track Scientist
Mast Cell Physiology Section	Dean Metcalfe, M.D., Section Head Parris Burd, Ph.D., Tenure-Track Scientist
Mucosal Immunity Section	Warren Strober, M.D., Section Head Stephen James, M.D., Tenure-Track Scientist
Bacterial Diseases Section	Harry Malech, M.D., Section Head John Gallin, M.D., Tenured Scientist Thomas Leto, Ph.D., Tenure-Track Scientist Daniel Rotrosen, M.D., Tenure-Track Scientist
Gene Therapy Unit	Karen Lomax, M.D., Tenure-Track Scientist
Clinical Mycology Section	John Bennett, M.D., Section Head Kyung-J. (June) Kwon-Chung, Ph.D., Tenured Scientist
Medical Virology Section	Stephen Straus, M.D., Section Head Jeffrey Ostrove, Ph.D., Tenure-Track Scientist

In August, 1990, Dr. Frank left the NIAID to become Professor and Chief of Pediatrics at Duke University. After a national search, Dr. Straus was appointed fourth LCI Chief in February, 1991. At the same time, Dr. Gallin created the Laboratory of Host Defenses under his own direction, and the task of NIAID Clinical Director was separated from that of LCI Chief and offered to Dr. H. Clifford Lane. LCI resources were reduced correspondingly; sections remained initially under the directions of Drs. Strober, Metcalfe, Kaliner, Bennett, and Straus (Figure 3).

The New LCI

In accepting the position of Laboratory Chief, Dr. Straus was asked to continue LCI's multi-disciplinary clinical and basic research efforts in allergy, immunology, and infectious diseases. Nonetheless, numerous changes were made in the LCI. Some of the changes were to accommodate expansion of the newly formed Laboratory of Host Defenses and the separate Office of the NIAID Clinical Director but, most importantly, to consolidate and redirect the research agenda of the LCI itself.

Changes in the LCI were made according to four basic principles. First, the existing LCI sections had grown relatively large and resources needed to be appropriated to accommodate and recruit new young scientists. Second, presaging the newly promulgated NIH-side guidelines for tenure-track scientists, the reality and wisdom of sustaining and supporting all of the, then, "tenure-track" scientists in the LCI was reviewed and firm commitments were made only to those

individuals for whom the resources, programmatic needs, and excellence would justify tenure track. Third, the components of the LCI had been relatively isolated one from another because there was little common ground for collaboration among groups engaged in such widely disparate research areas. If the Laboratory were to sustain efforts in allergy, immunology, and infectious diseases, new recruitment would best be made in disciplines that would bridge the existing LCI interests and draw them closer together. Fourth, there needed to be a major review of all on-going LCI projects and resource commitments to encourage growth toward areas of fundamental cellular and molecular biology and away from some of the more traditional biochemical approaches.

In keeping with each of these goals, changes were made in the staff and resource allocations in every Section of the LCI. Each of these is more fully delineated in the separate Section reports, so they will be summarized here briefly:

Office of the Chief

Under Dr. Straus' direction, a departmental library/conference room was recreated and renovated; several changes were made in administrative and secretarial support; and Mrs. Carol Penberthy was recruited as secretary and assistant to the Laboratory Chief. Dr. Straus initiated a practice of annual budget and programmatic reviews for each research group as well as extensive meetings with each of the Sections, in their entirety, to review all of the projects being carried out.

Clinical Immunology Section

With Dr. Frank's departure, the position of Section Chief was left vacant. Dr. Carl Hammer moved to the NIAID facilities in Frederick, Maryland, to assume a position there in the Biological Resources Branch. The remaining Section resources were either reassigned or consolidated into a new program, the Bacterial Pathogenesis Unit, headed by Dr. Thomas Russo.

Bacterial Pathogenesis Unit

In 1991, Dr. Thomas Russo assumed the position of Head of this Unit. He was supported with approximately 450 sq. ft. of laboratory space, a technician, a postdoctoral Fellow, and occasional students. Dr. Russo remained in the tenure track. In mid-1993, his research accomplishments were formally reviewed by an *ad hoc* panel of three outside experts, and it was deemed unlikely that he would attain scientific tenure within NIAID by the time the completion of his seventh year, in 1996. Dr. Russo was afforded one additional year of support to conclude his research here and to identify a new position elsewhere. Dr. Russo has always been an exemplary colleague and one of the most outstanding clinicians and teachers, so this decision was particularly difficult.

Allergic Diseases Section

In 1991, the commitment made by Dr. Frank to present Dr. Hohman for scientific tenure was honored by Dr. Straus, but tenure was not awarded, and Dr. Hohman was afforded one year to complete his work. He has assumed an independent research position in a nearby biotechnology company.

In August, 1993, Dr. Michael Kaliner announced his retirement from the United States Public Health Service after 20 years of service and leadership in the LCI. He had been Director of the Allergy/Immunology Training Program for most of that time, Director of the NIAID Asthma Center, and Head, Allergic Diseases Section. He has assumed directorship of a new, private asthma and allergy institute of his design affiliated with the Washington Hospital Center. He remains an indefatigable national proponent of allergy teaching and practice. Dr. Martha White departed with Dr. Kaliner to direct clinical and laboratory studies with him.
Dr. Dean Metcalfe has been proposed as the new Director, Allergy and

Immunology Program, and documents have been forwarded to have him replace Dr. Kaliner as Chief, Allergic Diseases Section, and to vacate the Mast Cell Physiology Section. Four of Dr. Kaliner's former Fellows have been reassigned until the completion of their training here.
One-third of the space vacated by Dr. Kaliner's group was designated for recruitment and support of Dr. Robert Seder (see below)

Mast Cell Physiology Section

As mentioned, Dr. Metcalfe will be moving administratively to occupy the position of Head, Allergic Diseases Section. Dr. Parris Burd departed the Mast Cell Physiology Section in 1992 to assume a research position in the FDA. Dr. Metcalfe's resources were expanded to strengthen both his basic and clinical research programs. He was assigned a second technician, afforded extended support for Fellows studying molecular aspects of mast cell biology, and a position was recently provided for him to initiate a new clinical asthma program under the day-to-day direction of Dr. Calvin Prussin.

Mucosal Immunity Section

Dr. Warren Strober was appointed Deputy Scientific Director, NIAID, in 1991 to add to his existing responsibilities as Section Head in the LCI. His colleague, Dr. Stephen James, left the LCI in 1991 to assume a position as Professor and Chief of Gastroenterology at the University of Maryland. Some of Dr. Strober's original research space was ceded to the Laboratory of Immunology in exchange for comparable adjacent space. All of Dr. Strober's space was renovated to his specifications in 1993.

For the past two years, a nationwide search has been under way to recruit an additional tenure-track scientist to initiate a new program within Dr. Strober's Section. The position was offered recently to a scientist from Harvard who studies lymphocyte development through a novel murine gene knockout model.

Clinical Mycology Section

The space occupied for many years by Drs. Bennett and Kwon-Chung at the end of the North corridor was ceded to the Laboratory of Host Defenses, and new space was designated for their use in the ACRF. In support of Dr. Bennett's position as Director, Infectious Disease Training Program, and as NIH's leading clinical infectious disease scholar, a new office was fabricated for him in the clinic near his laboratory.

The Section's move to new space provided an opportunity to reconfigure the relative distribution of resources for Drs. Kwon-Chung and Bennett. Dr. Kwon-Chung was afforded about a 50% increase in resources, equal now to that of Dr. Bennett, and a molecular mycologist, Dr. Yun Cheng, was hired as a Senior Staff Fellow to assist her in molecular studies of *Cryptococci* and to strengthen the molecular expertise of the Section as a whole. Dr. Bennett has been permitted to extend considerably the appointments of two excellent Fellows including Dr. Antonia Geber, who

remains in NIAID with independent grant funds through an appointment with George Washington University. Also, a research nurse was recruited to assist with Dr. Bennett's clinical research projects.

Medical Virology Section

With Dr. Frank's departure, this Section vacated its space and moved entirely to space originally occupied by Dr. Frank adjacent to the Laboratory's central offices. The new space increased the Section's allocation by about 40%. Dr. Jeffrey Ostrove left the NIH to assume a position in private industry.

Dr. Jeffrey Cohen was recruited from Dr. Elliott Kieff's laboratory at Harvard to join this Section as a tenure track candidate. His resources have expanded steadily in accord with his productivity. He now has 500 sq. ft. of his own space and shares all of the tissue culture and large equipment resources with Dr. Straus. Dr. Cohen is supported by a technician and three postdoctoral Fellows and has been invited to recruit a fourth. Dr. Cohen serves, on a rotating basis, as an attending physician on the NIAID inpatient units and on the NIH Infectious Disease Consult Service. In the summer of 1993, Dr. Cohen was formally reviewed and approved for tenure submission by the Board of Scientific Counselors during their site visit to the NIAID's Rocky Mountain Laboratories in Hamilton, Montana. Dr. Cohen's review by NIAID's Promotion and Tenure Committee will take place in April, 1994, and recommendations will have been forwarded to Dr. Gallin by the time of the May review of the LCI.

Additional resources, including a research nurse and a part-time Medical Officer and space for them in the Clinic, were assigned to Dr. Straus in support of his clinical research programs, particularly his extensive outpatient investigations of new treatments for hepatitis and recombinant vaccines for genital herpes.

Cytokine Biology Unit

Following a review of multiple staff candidates, Dr. Joshua Farber was recruited in 1993 to form a new research unit. Dr. Farber was an independently funded Assistant Professor of Medicine and Infectious Diseases at Johns Hopkins University, where he had been conducting studies of novel macrophage proteins which are synthesized and released in response to cell treatment with interferon-γ or LPS. Dr. Farber has been allocated about 750 sq. ft. of space and now has four postdoctoral Fellows. He will serve as an attending physician on the NIAID inpatient units on a rotating basis.

Lymphokine Regulation Unit

Also in 1993, Dr. Robert Seder was recruited to the LCI as a new tenure-track investigator. Trained in medicine at Cornell, Dr. Seder served four very productive years as a postdoctoral research Fellow with Dr. William Paul. His work primarily focused on *in vitro* and murine

studies of IL-4 and its ability to regulate the balance between Th1 and Th2 responses. In moving to the LCI, Dr. Seder will be completing the clinical requirements to make his eligible for Boards in Allergy/Immunology while he has established his own laboratory. He has been initially assigned about 400 sq. ft. of laboratory space, a technician, and two postdoctoral Fellows and has been given authority to recruit a third. It is intended that he will serve as a consultant and attending physician in the allergy/immunology clinical services. In the laboratory, he will pursue studies of lymphokine regulation in three model disease states. He has begun an exploration of IL-12 on histoplasmosis in mice in collaboration with Dr. Bennett and in HIV infection together with some of Dr. Fauci's staff. With a new Fellow, he is initiating a major analysis of T cell responses and lymphokine regulation in tuberculosis.

Clinical Studies Unit The complexity of contemporary clinical research demands a level of attention and expertise that no longer permits productive scientists to engage in it casually. To aid LCI staff in their ability to initiate and actualize novel studies of disease pathogenesis and therapy, a new Unit was established, originally headed by Dr. Michael Sneller, an outstanding clinician trained in rheumatology and infectious diseases. In 1993, however, he was recruited by Dr. Fauci to fill a staff vacancy in his own Laboratory. The Unit remains vacant.

Current Resources

The LCI occupies some 35 research modules ranging from 190 to 250 sq. ft. each; six additional office modules ranging from 75 to 250 sq. ft. are designated for clinical, clerical, and administrative support (Figure 3). There is now a renovated departmental library/conference room. The LCI has one small cold room in the 11C200 corridor; and it shares two other cold rooms on the floor with the Laboratory of Host Defenses, and two large equipment rooms with the Laboratory of Host Defenses and the Laboratory of Immunoregulation. Those large equipment rooms house most of the ultracentrifuges, beta and gamma counters, and a darkroom used by the LCI. Large common equipment items held within specific LCI Sections include two oligonucleotide synthesizers and two FPLC machines, and a new automated beta plate-reader has been purchased.

Given the departure of Dr. Kaliner and the expected departure of Dr. Russo, the LCI would have seven laboratory modules (approximately 1350 sq. ft.) of space uncommitted. The LCI has worked hard to create this space as an opportunity for one additional staff recruit and expansion of the space available as young scientists grow and flourish. In the interim, the available space will be loaned—on a temporary basis—to various LCI programs.

Operational Overview

Administration: Dr. Straus meets once or twice monthly with Dr. Gallin and all other Laboratory Chiefs and attends the annual Laboratory Chiefs' retreat. He meets monthly with the Administrative Officer assigned to the Laboratory and, periodically, with the Personnel Officer.

LCI Senior Staff, Administrative Officer, and Head Secretary meet monthly with Dr. Straus to review common issues of space, equipment, resources, recruitment, and budget.

Budget: Each Section/Unit develops its own budgetary proposal and reviews it formally with Dr. Straus each spring. The budget is compiled and adjusted by Dr. Straus and submitted to Dr. Gallin along with copies of each group's original request. Formal meetings with Dr. Gallin and then the Senior Staff occur each fall as the annual budget is allocated by Congress.

Personnel: Senior Staff, technicians, and research nurses are relatively fixed resources committed for prolonged time frames to each Section or Unit. Needs for postdoctoral Fellows are reviewed annually with Dr. Straus and committed to the Sections and Units based upon programmatic needs, worthiness of the project, and excellence of the applicant.

Science: Each Section or Unit present formal, written reports of its work and plans every spring. Dr. Straus meets formally with each group every fall to review their progress.

LCI Senior Staff and Fellows review their recent work in rotation at a research seminar held every Wednesday morning. Each group holds its own weekly research meetings, and most hold weekly journal clubs.

Fellows and staff are encouraged to present their work at national and international meetings, as the restricted travel budget permits. All Fellows are allocated funds for one domestic meeting per year.

Clinical staff round formally three times per week and participate in weekly NIAID Medical Grand Rounds and subspecialty conferences.

Clinical Program

Nearly all of LCI's staff are clinically trained and participate in patient care, teaching, and clinical research. Fourteen of LCI's 41 postdoctoral Fellows are in the training programs leading to eligibility for subspecialty boards in Allergy/Immunology or Infectious Diseases.

The LCI staff admit approximately one-fourth of all patients in the 11th floor nursing units and account for 80% of the nearly 2,500 annual visits to the 11th floor clinic.

Clinical Fellows and Physician Assistants carry out much of the primary inpatient care. LCI Fellows and a cadre of five research nurses and one Medical Officer provide primary care, data collection, and monitoring in the outpatient clinic, all under the direct supervision of LCI Senior Staff.

Major current LCI clinical projects are engaged in studies of the pathogenesis of immunoglobulin deficiency disorders, common variable immunodeficiency, inflammatory bowel disease, mastocytosis, chronic viral and fungal infections, recurrent urinary tract infections, polyclonal lymphoproliferative disorders, and chronic fatigue syndrome. Novel therapies are being explored for mastocytosis, hepatitis, zoster, and candidemia, while vaccines in clinical development in the LCI are directed against genital herpes and cryptococcoses.

NIH-Wide and National Commitments

LCI staff comprise most of the NIH's clinical allergy and much of its clinical infectious diseases expertise. Dr. Metcalfe directs the NIH accredited training program in Allergy and Immunology. Dr. Bennett directs the accredited Infectious Diseases fellowship program. All of the LCI's Senior Staff, excepting Dr. Kwon-Chung, provide clinical service and teaching. LCI staff also serve on many major national and NIH committees and policy boards, including the Recombinant DNA Advisory Committee, the AIDS Data Safety and Monitoring Board, the Infectious Diseases Society of America, the Clinical Immunology Society, the American Academy of Allergy and Immunology, and the American Board of Allergy and Immunology.

Honors and Awards

Drs. Strober, Metcalfe, and Straus are elected members of the American Society for Clinical Investigation and the Association for American Physicians. Dr. Bennett was awarded the title of Master of the American College of Physicians in 1991. Dr. Kwon-Chung is recipient of numerous awards including the prestigious Sung Ji Academy Award of Korea in 1992.

LCI staff serve on editorial boards of *The Journal of Immunology, The Journal of Clinical Immunology, Clinical and Experimental Allergy, Antimicrobial Agents and Chemotherapy,* and *Virology*. Dr. Strober edits *Current Protocols in Immunology*; Dr. Bennett is founding editor of the award-winning textbook *Principles and Practices of Infectious Diseases*. Dr. Straus is an associate editor of *Field's Virology*. Dr. Kwon-Chung is an editor of two major textbooks of medical mycology.

The Future

The LCI has been redirected into a group of smaller programs, including three by dynamic young tenure-track investigators. Recruitment of Dr. Farber to study macrophage gene products brought to the LCI an infectious disease-trained investigator whose bench interests closely relate to those of Drs. Strober and Metcalfe. Dr. Seder, an immunologist, is studying lymphokine responses to three microbes. These initiatives succeeded in drawing potentially disparate groups of the LCI more closely into a cohesive entity.

In January, 1994, however, the Institute committed to form a new intramural Laboratory of Allergic Diseases and to house it, at least in part, in the remaining space vacated by Dr. Kaliner (1000 sq. ft.) and all of the space currently occupied by Dr. Metcalfe (900 sq. ft.). This will

serve to remove all of the allergy research component from the LCI. This demands a further redirection of the LCI program and resources. Additional staff recruitment, now, can only take place upon the retirement or departure of existing LCI staff. The laboratory effort will, hereafter, center solely around fundamental and clinically important aspects of microbial and immunopathogenesis.

It is expected that the growth and improved productivity of the young, tenure-track scientists in the LCI will justify increasing the resources of one or more of them in the coming three to four years. To serve Dr. Cohen's needs, additional space currently occupied by Dr. Straus' staff will be made available to him. For the others, there remains approximately 600 sq. ft. of "reserve" space in the LCI after creation of the new Laboratory of Allergic Diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01Z10057-21-LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on Pathogens Causing Cryptococcosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

K.J. Kwon-Chung, Ph.D. - Research Microbiologist - Clinical Mycology Section, LCI, NIAID
Others: Yun C. Chang, Ph.D. - Senior Staff Fellow - Clinical Mycology Section, LCI, NIAID
Ashok Varma, Ph.D. - Microbiologist - Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH - Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Molecular dissection of the genes responsible for the formation of extracellular polysaccharide capsule in Cryptococcus neoformans, a major opportunistic fungal pathogen causing infection primarily in immunocompromised patients. The extracellular polysaccharide capsule of C. neoformans has been the major virulence factor of the organism. To understand the molecular aspects of capsule synthesis in C. neoformans, we attempted to clone the genes controlling the formation of polysaccharide capsule in the fungus. A wild type genomic DNA library of C. neofmans B-3501 was used to complement various cap mutants. The plasmids containing appropriate DNA inserts from Cap transformants was rescued in E. coli. Subsequent complementation of various Cap mutants with the subclones of the insert DNA's indicated that we have cloned the gene, Cap59. To demonstrate that the Cap59 gene is essential for the virulence of C. neoformans, the gene was deleted from a wild type strain and tested for its' virulence. The Cap59 deleted strain became avirulent in mice while the wild type strain produced fatal infection within 20 days in all 10 (100%) mice. The gene deletion by homologous integration presented in this study is the first such report in C. neoformans. A linear minichromosome of 270kb in size was found to have been created in a C. neoformans ura5 mutant as a result of transformation with a plasmid containing C. neoformans URA5 gene. The minichromosome was as stably maintained as native chromosomes during mitotic or meiotic cell cycle. Analysis of the minichromosome showed that is contains at least four copies of URA5 sequence and 23 copies of rDNA clusters in addition to unknown sequences. Analysis of the subclones of the unidentified DNA showed that there are several repeats of the conserved core of the ARS-like (autonomously replicating sequences), sequences [A/TTTTAT(A/G)TTT(A/T)] within a 4.5kb fragment. These sequences were suspected to be the sequences contributing to its' apparent stability by the maintenance of high copy numbers of the host plasmid. The arrangement and sequence of the 5S rRNA in C. neoformans was determined. The gene is composed of 118 nucleotides and is located at 1kb upstream of the small subunit rRNA gene within the rRNA cluster. The 5S gene was found to be transcribed at the same direction as is the case of the 5S gene in the majority of the higher basidiomycetous fungi.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 0058-20 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis and Chemotherapy of Herpesvirus Infection in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus, Senior Investigator, LCI, NIAID

Others: J. Cohen, Senior Staff Fellow, LCI, NIAID
R. Kost, Medical Staff Fellow, LCI, NIAID
J. Meier, Medical Staff Fellow, LCI, NIAID
J. Lekstrom-Himes, Medical Staff Fellow, LCI, NIAID
Y. Yi, Visiting Fellow, LCI, NIAID
R. McKenzie, Medical Staff Fellow,LCI, NIAID
T. Heineman, Medical Staff Fellow,LCI, NIAID
J. Dale, Research Nurse, LCI, NIAID
M. Nakamura, Medical Staff Fellow,LCI, NIAID
K. Wang, Visiting Associate, LCI, NIAID

COOPERATING UNITS *(if any)*

A. Notkins, Chief, LOM, NIDR; L. Stanberry, University of Cinncinnati; P. Krause, CBER/FDA; R. Hunziker, Transgenic Mouse Facility

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.4	4.5	0.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to characterize the pathogenesis, natural history and therapy of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections. Our clinical emphasis has been on oral and genital herpes and zoster in the normal and the immuno-compromised host. Over the years we established the value, long term efficacy and safety of oral acyclovir for suppression of recurrent genital herpes and more recently oral herpes as well. We initiated collaborative studies of BVaraU, a new drug for zoster. Also in the past year, we studied and reported the first known instance of a persistent acyclovir-resistant HSV2 infection in an immunologically normal individual.

The major basic research thrust of this laboratory has been to define molecular aspects of SHV and VZV latency and pathogenesis. We are examining the role of the HSV I and II latency-associated transcripts (LAT) in control of virus latency and reactivation. Recombinant virsues deleted for LAT expression are being studied in vitro and in animal models. We have begun to create transgenic mice expressing HSV genes neighboring and/or including the LAT gene. We are also studying specific LAT promotor elements.

Work on VZV latency and gene regulation has concentrated on genes 4, 10, 28, 21, 61, 62, and 63. Because gene 29 is expressed in latency and 28 is not, we are studying the regulation of these two genes. We found that they share a common expression of both genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00249-13 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID

Others: Alexandra Worobec, M.D. Medical Staff Fellow, LCI/NIAID

Hiroshi Nagata, M.D., Ph.D., Fogarty Visiting Fellow, LCI/NIAID

Arnold Kirshenbaum, M.D., Special Volunteer, LCI/NIAID

COOPERATING UNITS *(if any)*

Laboratory of Host Defense Mechanisms, NIAID Dr. John Gallin

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.80	2.55	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mast cell precursor in human peripheral blood is $CD34^+/Fc_{\varepsilon}RI^-$, gives rise to mast cells in the presence of recombinant human stem cell factor (SCF) with or without IL-3, and the number of mast cells arising per $CD34^+$ cell in culture is greater when the $CD34^+$ cells are obtained from patients with mastocytosis compared to normal subjects.

The degree of abnormality on initial bone scan and progression of scintigraphic abnormalities with serial scanning in patients with mastocytosis correlate with the presence of more aggressive systemic mastocytosis. In many cases this may be a reflection of bone marrow expansion, which in turn probably reflects increased marrow disease.

An analysis of cytokine profiles in dermal suction blisters has demonstrated that mastocytosis patients over-produce TNF-α.

Mast cell tryptase in patients with mastocytosis differs from tryptase released during anaphylaxis. This difference is recognized by specific monoclonal antibodies and may allow the development of a more specific diagnostic test for mastocytosis.

Studies are underway to examine c-kit for point mutations that may relate to the etiology of mastocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00354-12 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Warren Strober, M.D.	Chief, MIS Section	LCI, NIAID
Ivan J. Fuss, M.D.	IRTA Fellow, MIS	LCI, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of inflammatory bowel disease (IBD) suggest that the basic abnormality consists of an unrestrained immunologic response, not to novel antigen, but rather to common antigens to which the mucosal system is ordinarily unresponsive. To gather data relevant to this hypothesis we have performed extensive studies of control lamina propria T cells. In initial studies we established that as compared to control peripheral blood T cells, lamina propria T cells manifest a greatly reduced response to stimulation via the TCR/CD3 pathway. This was true when cells were studied as a relatively unpurified form or when examined as highly purified CD4+ T cells. Responses via alternative pathways, i.e., via CD2 ± CD28 were also reduced, but not nearly so reduced as via TCR/CD3. In addition, the unresponsive state could be at least partially reversed by cultured cells in IL-2 alone (not IL-2 plus proliferative stimulus) for 24 hours; this suggested that the unresponsiveness was a form of reversible anergy. In further studies, we examined the capacity of peripheral blood and lamina propria T cells to produce various cytokines under the above stimulation conditions. Here we found that both peripheral blood and lamina propria T cells produced relatively low amounts of IL-2, as well as IFN-γ or Il-4, when stimulated via the TCR/CD3 pathway alone, but produced large amounts when stimulated via the CD2/CD28 co-stimulatory pathway. Thus, despite the fact that lamina propria T cells manifest greatly reduced proliferation, they produce as much or more cytokines, as compared to peripheral blood T cells. Finally, we performed studies to elucidate the mechanism of this form of "split" unresponsiveness. In particular, we cross-linked TCR/CD3 T cells under stringent conditions and did indeed induce T cell unresponsiveness (to subsequent stimulation via the TCR/CD3 pathway). However, such cells were also no longer responsive via the CD2 pathway either by proliferation or cytokine production. Thus, the unresponsiveness/responsiveness of lamina propria cells cannot yet be reproduced in culture. Overall, these studies reveal that lamina propria T cells achieve a unique functional state which allows them to act as effector cells in the local mucosal environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00 356-12 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Regulation of IgA Immunoglobulin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Warren Strober, M.D.	Chief, Mucosal Immunity Section, LCI, NIAID
Other:	Brian L. Kelsall, M.D.	NRSA Fellow, MIS, LCI, NIAID
	Rolf Ehrhardt, M.D.	Visiting Fellow, MIS, LCI, NIAID
	Markus Neurath, M.D.	Special Volunteer, MIS, LCI, NIAID
	Milan Basta, M.D.	Visiting Associate, MIS, LCI, NIAID
	Eckhard Stuber, M.D.	Special Volunteer, MIS, LCI, NIAID
	Belinda Gray, M.A.	Biologist

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project 1: In studies of IgA B cell differentiation in Peyer's patches we have obtained evidence that a major reason for preferential IgA B cell development at this site is due to enhanced survival of post-switch IgA B cells. In particular, we have shown that Peyer's patch cell populations entirely depleted of sIgA+ B cells by cell sorting technique, support the survival and differentiation of purified sIgA+ B cells, whereas spleen cell populations fail to do so. In other studies of IgA B cell differentiation, we have shown that highly differentiated IgA B cells (and IgG B cells) respond poorly to stimulation by agents that cross-link the Ig receptor (a one-signal event) but respond well to stimulation by T cells (a multi-signal event). In fact, cross-linking of IgA (with anti-IgA-dextran) on sIgA+ B cells abrogates subsequent stimulation of the B cells by T cells: anti-IgA-dextran "tolerizes" IgA B cells. Such tolerization is, however, inhibited by co-stimulation of cells with CD40L. **Project II:** In molecular studies of B cell differentiation, we have shown that B cell-specific activation protein (BSAP) binds to a site in the 3'α enhancer and that such binding downregulates enhancer function as detected in studies of transfected plasmids containing the enhancer linked to a reporter gene (luciferase gene). In parallel studies we showed that downregulation of BSAP with anti-sense oligonucleotides leads to markedly decreased B cell proliferation, whereas upregulation of BSAP with BSAP-producing plasmid leads to increased B cell proliferation. Taken together, these results support the concept that BSAP promotes B cell growth and differentiation while it inhibits plasma cell development and Ig secretion. **Project III:** As part of a broad study of T cell differentiation in Peyer's patches (PP), we have studied PP dendritic cell capacity to present antigen (cytochrome C) to T cells from mice expressing a cytochrome C-specific TCR transgene. We showed that PP dendritic cells are phenotypically distinct from spleen dendritic cells (express more MHC class II) and in both primary and secondary culture systems induce T cells to produce 5-10-fold more IFN-γ than spleen dendritic cells. This process is IL-2-dependent and inhibitable by IL-4. The capacity of PP dendritic cells to induce T cells to produce IFN-γ is less evident in highly purified dendritic cells isolated by sorting on the basis of N418 antigen expression. These results indicate that PPs contain a phenotypically and functionally unique dendritic cell population that leads to TH1-type T cell responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00430-10 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Varicella-Zoster Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.I. Cohen - Senior Staff Fellow, LCI, NIAID
S.E. Straus - Chief, LCI, NIAID
J. Meier - Clinical Associate, LCI, NIAID
R.Kost - Clinical Associate, LCI, NIAID
H. Moriuchi - Fogarty Fellow, LCI, NIAID
K.E. Seidel - Bio. Lab. Tech., LCI, NIAID
M. Moriuchi - Bio. Lab. Tech., LCI, NIAID

COOPERATING UNITS *(if any)*

M. Sawadago, M.D. Anderson Cancer Center
S. Triezenberg, Michigan State University

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	2.6	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary infection with varicella-zoster virus (VZV) causes chickenpox, and reactivation of the virus from latency results in zoster. The goals of this project are to identify and determine the function of VZV genes that are expressed during active infection and during latency in the body.

We are currently investigating the functions of VZV genes that regulate viral gene expression. Analysis of VZV gene products during acute infection indicate that at least five VZV genes, ORF4, ORF10, ORF61, ORF62, and ORF63, transactivate the expression of other VZV genes in vitro. Two of these genes (ORF10, ORF62) activate transcription directly and their activation domains have been identified. Three of these VZV genes (ORF10, ORF61, and ORF62) can complement the function of their herpes simplex virus homologs, while one of the genes (ORF4) cannot. Chimeric proteins, containing portions of VZV proteins and their HSV-1 homologs, have been constructed to compare functional domains of these proteins.

Two VZV genes, ORF28 and ORF29, have bidirectional, overlapping promoters that regulate their expression. These control elements are activated by a viral protein (ORF62) and a cellular protein termed USF. While both of these genes are expressed in acute infection, one of the genes (ORF29) is expressed during dormant infection, while the other gene (ORF28) is not.

Analysis of trigeminal ganglia from human cadavers without active evidence of VZV infection indicates that at least two VZV genes (ORF29 and ORF62) are expressed during viral latency, while other genes (ORF10, ORF28, and ORF61) are not.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00 432-10 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of immune responses in humans and non-human primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

P.I.: Warren Strober, M.D. Chief, Mucosal Immunity Section, LCI, NIAID

Other: Atsushi Kitani, M.D., Visiting Fellow, MIS/LCI/NIAID
Chingchai Wanidworanun, M.D. Visiting Associate, MIS, LCI, NIAID

COOPERATING UNITS *(if any)*

N/A

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

I) To further elucidate B cell isotype differentiation in humans, we investigated the regulation of germline and mature Cα mRNA transcript expression in human peripheral blood B cells. Focusing first on regulation of germline (Iα-Cα) transcripts (GLT), we showed that Cα1 GLT are induced by TGF-β alone, but such induction is enhanced by *S. aureus*, Cowan 1 (SAC). In contrast, Cα2 GLT are optimally induced by TGF-β1 in the absence of a B cell stimulus. Turning next to transcripts, and such induction is IL-10-dependent because it is enhanced by exogenous IL-10 and abrogated by the presence of anti-IL-10. In contrast, Cα2 transcripts are induced by T cell stimuli such as anti-CD40 (presented by CDw32-transfected L cells) in the presence of TGF-β1/IL-10 or by PWM-activated T cells. In summary, these studies show that Cα1 and Cα2 germline and mature transcripts induction is differentially regulated. In addition, this shows that IL-10 appears to be uniquely supportive of the induction of both Cα1 and Cα2 mature transcripts.
II) IL-10 has been shown to play an important role in inflammation. To further investigate this property, we determined if polymorphonuclear cells (PMNs) are similar to other phagocytic cell types in their capacity to produce IL-10. Accordingly, human PMNs were isolated from buffy coat preparations and then cultured in the presence of absence of LPS; resulting IL-10 production was assayed at the mRNA level using RT-PCR and at the protein secretion level using IL-10-specific ELISA. We found that after 24 h of incubation, LPS-stimulated PMNs produced a substantial amount of IL-10 mRNA and secreted IL-10 protein (600-3000 pg/ml). Such production, however, was not seen in highly purified PMNs unless mononuclear cells were added back; thus, it is likely that another cell population secretes a factor which induces stimulated PMNs to produce IL-10. Finally, we showed that the above production of IL-10by PMNs has potential self-regulatory effects: LPS-induced expression of the adhesion molecule Mac-1 is suppressed by IL-10 and enhanced by anti-Il-10. In addition, antibody to IL-10 caused a 3-10-fold enhancement of TNF-α, IL-1α and IL-10 mRNA and/or protein production. These studies support the view that PMNs are a major source of IL-10 at inflammatory sites and IL-10 is a self-regulator of PMNs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00470-09 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chronic Epstein Barr Virus Infection and Chronic Fatigue Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Straus - Senior Investigator, LCI, NIAID

J. Dale - Clinical Research Nurse, LCI, NIAID

E. Benson-Grigg - Guest Research Nurse, LCI, NIAID

R. McKenzie - Medical Officer, LCI, NIAID

M. Sneller - Medical Officer, LIR, NIAID

J. Cohen - Senior Staff Fellow, LCI, NIAID

J. Lekstrom-Himes - Clinical Assoc.,LCI, NIAID

A. O'Fallon - Clinical Res. Nurse, LCI, NIAID

W. Strober - Senior Investigator, LCI, NIAID

COOPERATING UNITS *(if any)*

J. Grafman (LNM, NINCDS), H. Rotbart (Univ. of Colorado), F. Hayden (Univ. of Virginia) M. Demitrack (Univ. of Michigan), D. Garcia-Borregeros,M. Altemus, P. Gold (NIMH), P.Peeke, D. Garcia, G. Chrousos (NICHD), Scott Fritz (PRI, FCRF), E. Jaffe, J. Fleischer (CC, NIH)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	3.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this project are to characterize severe chronic infections with Epstein Barr Virus (EBV) and other lymphoproliferative disorders, and to elucidate multiple aspects of the chronic fatigue syndrome which was, earlier, considered to be related to EBV infection. To date this research project has involved about 250 patients. Included are 7 patients who were diagnosed with severe chronic EBV infections on the basis of clinical, histological, molecular and serologic features. We continue to examine immunologic features of patients with severe chronic EBV-associated lymphoproliferation and explore treatments. Acyclovir, alpha and gamma interferons proved of little value, but immunosuppressive therapies are being used with good long-term results.

Detailed immunologic, neurologic, endocrinologic and psychologic studies are being conducted on selected patients with chronic fatigue. To date, we still have no consistent laboratory abnormality that permits a clear diagnosis of the chronic fatigue syndrome, however, we have been pursuing the basis and meaning of group abnormalities in neuropsychiatric, immune and endocrine sysems. A series of studies of the pituitary-adrenal responsiveness to corticotropin releasing hormone and ACTH and of neuropeptide and catechol levels in spinal fluid suggest deficient central CRH release. Since CRH induces CNS arousal, these neuroendocrine findings suggest a new mechanism whereby the lethargy of Chronic Fatigue Syndrome patients may be explained. We are pursuing these observations in a new series of studies and a fresh patient cohort. Arginine-Vasopression infusions have been given to 24 patients and 20 controls to stimulate and test the HPA axis. The results will be available in late FY94. Forty-five patients were enrolled already in a placebo-controlled trial of hydrocortisone treatment. It should permit us to test the hypothesis that corticosteroid deficit leads to symptoms. We also recognized discrete abnormalities in CFS patient lymphocyte phenotype and in vitro responsiveness to mitogens in patterns suggesting mild immune activation. There is a reduction in naive (CD4xCD45RA) t Cells and an increase in memory (CD4xCD45RO) T cell bearing adhesion markers (LFA3, CD29, etc.). We have begun to pursue these findings in several other laboratory studies involving purified cell populations from patients and controls and in patients with acute influenza. A vigorous, multidisciplinary approach to the syndrome continues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00513-07 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Mast Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID

Others: Peter Bianchine, M.D. Medical Staff Fellow, LCI/NIAID
Jaroslaw Dastych, Ph.D. Fogarty Visiting Fellow, LCI/NIAID
Susetta Finotto, Ph.D., Fogarty Visiting Fellow, LCI/NIAID
Arnold Kirshenbaum, M.D., Special Volunteer, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.90	3.15	0.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mast cells adhere to fibronectin following aggregation of high affinity receptors for IgE ($Fc_{\varepsilon}RI$) on the membrane surface. This process is mediated by an alpha-5 containing integrin. $Fc_{\varepsilon}RI$-induced adhesion is transient (compared to stem cell factor-induced adhesion), and occurs through "inside-out signaling" which results in a conformational change in the surface integrins but does not alter their number.

Mast cells spontaneously adhere to vitronectin. This event is followed by the phosphorylation of multiple intracellular proteins, a process which can be detected as early as 5 minutes after adhesion. Among the phosphorylated proteins is focal adhesion kinase (FAK). FAK is also phosphorylated after aggregation of $Fc_{\varepsilon}RI$, or after the addition of c-kit ligand, also termed stem cell factor (SCF).

IL-3 dependent mast cells undergo apoptosis (programmed cell death) following removal of IL-3. This is prevented by the addition of SCF. Addition of TGF-beta prevents this SCF-mediated rescue, thus demonstrating one way in which the microenvironment can control mast cell numbers.

Mouse bone marrow-derived mast cells respond to both IL-3 and c-kit ligand and are inhibited by m-CSF, GM-CSF, and γ-IFN. Mouse peripheral blood mononuclear cell-derived mast cells respond only to c-kit ligand.

Human peripheral blood-derived human mast cells are derived from a $CD34^+$ $Fc_{\varepsilon}RI^-$ cell population. CD34 positivity is rapidly lost in culture, while the cell population becomes $Fc_{\varepsilon}RI^+$. The culture of human mast cells is enhanced if human serum is used instead of fetal calf serum.

Fibroblasts in vitro selectively support the survival of mast cells, while internalyzing and degrading basophils.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 0000548-06 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters of less. Title must fit on one line between the borders.)*

Prevention of Genital Herpes Simplex Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E. Sraus - Senior Investigator - LCI, NIAID

R. McKenzie - Medical Officer - LCI, NIAID

B. Savarese - Research Nurse - LCI, NIAID

R. Kost - Medical Staff Fellow - LCI, NIAID

T. Heinemen - Medical Staff Fellow - LCI, NIAID

J. Lekstrom-Himes - Medica.l Staff Fellow - LCI, NIAID

M. Nakamura - Medical Staff Fellow - LCI, NIAID

E. Cox, Medical Staff Fellow - LCI, NIAID

COOPERATING UNITS *(if any)*

R.L. Burke, M. Tigges, A. Langenberg, C. Dekker, (Biocine, Inc., Emeryville, CA)

L. Corey, (University of Washington, Seattle)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Having completed over a decade of work on antiviral treatment of genital herpes, we turned to studies of disease prevention and immunotheraphy. We completed an open 2 year phase 1 assessment of a new recombinant HSV-2 glycoprotein D vaccine in alum in 24 adults, some with and without prior HSV-1 and/or 2 infection. Vaccinations of 30 ug or 100 ug were given at 0, 1, 2, and 12 months and were associated with minimal local or systemic reactions. The vaccine induced excellent primary antibody in cellular immune responses and augmented preexisting humoral andc cellular responses signficiantly. We studied antibody and T cells from previously uninfected subjects and showed that the gD2 epitopes that the antibody and cell recognize correspond to those in genital herpes patients. On the basis of these excellent responses we enrolled patients with recurrent genital herpes into a placebo-controlled vaccine trial in collaboration with the University of Washington. The goal was to determine whether boosted immunity leads to less frequent recurrences. Upon completing the study we documented that of 98 subjects, those who received the gD2 vaccine in alum experienced 1/4 - 1/3 fewer recurrences than those receiving alum alone. This is the first ever trial to demonstrate efficacy in the immunotherapy of a chronic viral infection. Rates of disease improvement, though, were lower than would be expected with acyclovir suppressive therapy so we began a dose seekin study and an efficacy study using gD2 combined with gB2 in a lipid adjuvant MF59. This was completed in FY1993. Based on the data we initiated 2 further controlled trials, one for immunotheraphy, the other for prophylaxis. Over 110 patients were vaccinated thus far and will be followed until 1995.

PROJECT NUMBER

Z01 AI 00589-05 LCI
Project Terminated

PERIOD COVERED

October 1, 1993 to July 28, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Identification of Complement Resistance Determinants in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D., C.M., Senior Staff Fellow, NIAID, LCI
Charles Brown, M.S., Biologist, NIAID, LCI
Geetika Sharma, M.D., Visiting Associate, NIAID, LCI

COOPERATING UNITS *(if any)*

Alan Cross, M.D., Walter Reed Army Institute of Research, Washington, D.C.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.05	.85

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Certain strains of E. coli causes extraintestinal disease, ranging from cystitis to sepsis. Complement resistance appears to be a vital trait in the pathogenesis of infections by these strains. An improved understanding of the complement resistance determinants in this group of E. coli may to a logical development of new preventative and/or therapeutic strategies.

The experimental approach taken for this project was to study a serum resistant, non-K1, blood isolate as a model pathogen; as these serotypes are responsible for the majority of adult disease caused by extra-intestinal isolates of E.coli. Transposon mutagenesis (TnphoA) was utilized to create a library of mutants in outer, periplasmic and cytoplasmic membrane proteins. Mutants were screened for an increased sensitivity to serum. Isogenic group 2 (K54 antigen) capsule deficient mutants were identified with an increased sensitivity to serum, undergoing a 3-4 log kill over 3 hours in 80% serum. Furtherin vitro evaluations established that the K54 capsular polysaccharide protects against killing by the alternative complement pathway. In addition, C3 binding was equivalent in both strains. Therefore, the mechanism of complement resistance conferred by the K54 capsule is different than that of K1.

The E. coli LPS has been purported to be the major complement resistance determinant. Therefore, we undertook and were sucessful in creating proven isogenic LPS-minus (O specific chain) derivatives of CP9 as well as isogenic CP9 derivatives deficient in both the group 2 capsule (K54) and the O specific chain of the LPS (04). The results of our evaluations of these mutants, however, are in contrast to those of previous reports. The 04 antigen appears to play only a minor role in protecting against complement mediated killing. However, the loss of both the K54 antigen and the 04 render the strain extremely serum sensitive.The results of these studies expands on the understanding of complement resistance mechanisms in E. coli. This project is being terminated at N.I.H.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00590-05 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Primary Immunodeficiency Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Warren Strober, M.D., Chief, Mucosal Immunity Section/LCI/NIAID

Others: Thomas A. Selvaggi, M.D. Clinical Associate (NSF), Mucosal Immunity Section/LCI/NIAID

Eli M. Eisenstein, M.D., Clinical Associate, Mucosal Immunity Section/LCI/NIAID

Kevin Chua, B.S. Biologist, Mucosal Immunity Section/LCI/NIAID

COOPERATING UNITS *(if any)*

Laboratory of Host Defenses, NIAID (Harry L. Malech, M.D.)
Laboratory of Immunoregulation, NIAID (John H. Kehrl, M.D.)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892-0010

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies in our laboratory have focused on defining the nature of the B cell and T cell defects in Common Variable Immunodeficiency (CVI), a heterogeneous group of primary acquired human immunodeficiency states characterized by hypogammaglobulinemia and impaired functional antibody responses, at the cellular level, and identifying molecular mechanisms responsible for these defects. Studies of purified B cells of patients with CVI show that although the cells have a normal capacity to proliferate, they manifest differentiation defects at multiple levels. Compared with controls, circulating CVI B cell populations contain reduced numbers of sIgG+ and sIgA+ cells with a commensurate increase in sIgM+ B cells, suggesting an *in vivo* defect in isotype switch. In addition, CVI B cells manifest Ig secretion defects on stimulation. These Ig secretion defects are not overcome by addition of a variety of cytokines. In further studies we show that despite the above abnormalities, CVI B cells are induced to express normal or near-normal levels of $C\mu$, $C\gamma$, and $C\alpha$ mRNA after 7 days of stimulation with anti-CD40 and IL-10. That this C_H mRNA expression represents a recovery of CVI B cell differentiation is supported by studies of Ig secretion in which CVI B cells that are first stimulated for 7 days with anti-CD40 and IL-10 and then restimulated in coculture with activated normal allogeneic T cells and IL-10, secrete substantial levels of IgM and IgG and increased amounts of IgA. Overall, therefore, CVI B cell function can be significantly improved by maintenance in culture. These data suggest the abnormalities of B cell differentiation in CVI are reversible and that the defect is a form of B cell anergy. Commensurate with this hypothesis, we have done further experiments whose preliminary results, in fact, indicate that there is a defect in the ability of CVI B cells to act as antigen presenting cells. A second project focuses on X-linked agammaglobulinemia (XLA), another major humoral immunodeficiency syndrome. In affected individuals, B cell development is arrested at the pre-B cell stage with absence of mature B cells, resulting from mutations in a gene termed btk whose product normally exhibits tyrosine kinase. In collaboration with others, we have begun working toward gene therapy of XLA. Presently, we have developed a retroviral construct with the btk gene and confirmed its successful transfection into NIH 3T3 fibroblast cells by polymerase chain reaction (PCR).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00607-04 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cytokine Gene Expression in Mast Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID

Other: Chad Oh, M.D. Medical Staff Fellow, LCI/NIAID

Yoseph Mekori, M.D. Fogarty Visiting Scientist, LCI/NIAID

Jaroslaw Dastych, Ph.D. Fogarty Visiting Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

NCI/FCRDC (Oppenheim)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.50	1.60	0.90

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Northern blot analysis shows that TCA3 is expressed after activation with either PMA/ionophore or high affinity IgE cross-linking in C57 cells. mRNA of TCA3 is induced within 2 hours, peaks at 3 hours, and disappears at 6 hours. Half-life of mRNA determined by adding actinomycin D is approximately 1/2 hour. These results demonstrate that TCA3 is regulated transcriptionally. Nuclear run-on assays show that the rate of TCA3 mRNA transcription is increased after activation consistent with transcriptional regulation. Promoter analysis confirmed that the TCA3 gene is regulated transcriptionally in mast cells, and that the minimal promoter sequences are contained within the 0.082 kb upstream region of the TCA3 gene with a putative enhancer NF-kB element between -0.324 kb and -0.136 kb and a putative inhibitory element between 1.324 kb and 2.0 kb upstream from the transcription start site.

Functional assessment of the region between 1.324 kb and 2 kb demonstrated that there are at least two inhibitory elements. One is orientation independent.

Comparative analysis of the promoter region of TCA3 in T cells and mast cells revealed different patterns of regulation.

Direct mast cell-to-T cell interaction is required to induce transfected TCA3 CAT constructs in mast cells. Soluble mediators released by either unstimulated mast cells or cells stimulated by $Fc_{\varepsilon}RI$ cross-linking are not able to induce TCA3 CAT gene expression. Unstimulated mast cell pellets also are unable to direct expression of TCA3 CAT expression.

Tissue distribution of TCA3 mRNA is limited to skeletal muscle in the normal mouse. No message was detected in the brain, liver, lung, spleen, heart, or kidney. I-309 (human homolog of TCA3) was also not detectable in those organs and lymph nodes.

Mast cells chemotax to the chemokines RANTES and MCAF in a dose-dependent manner. Parallel studies demonstrate that this chemotaxis is not accompanied by mast cell histamine release. When mast cells are activated through $Fc_{\varepsilon}RI$, chemotaxis can also be demonstrated to MIP-1α, and PF-4.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00617-04 LCI

PERIOD COVERED

October 1, 1993 to July 28, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Capsule Synthesis in a Pathogenic Escherichia coli Isolate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D., C.M., Senior Staff Fellow, NIAID, LCI

COOPERATING UNITS *(if any)*

Susan Gottesman, Ph.D., Senior Investigator, LMP, NCI, NIH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antimicrobial therapy has not effectively minimized the significant morbidity and mortality associated with infections due to extra-intestinal isolates of Escherichia coli. Optimal therapy hinges on understanding their pathogenesis. Capsular antigens of E. coli have been variably suggested as being important virulence factors, with established serotypes being broadly categorized into groups 1 or 2. Date from our laboratory has established the group 2 capsule K54 as being an important virulence factor. Therefore, we began an evaluation of the regulationof the group 2, K54 capsular polysaccharide. As a starting point, we assessed the role of the group 1 capsule regulators RcsA,RcsB and Lon on the regulation of group 2 capsule production.

We have established the CP9, an extra-intestinal isolate of Escherichia coli can be induced to produce a group 1 capsular polysaccharide in addition to its constitutive production of a group 2, K54 capsule. The group 1 positive regulators, RcsA and RcsB, and the negative regulator Lon protease are present and act on group 1 capsule production as previously described for K-12 and K30 strains. The effect of these regulators on the group 2, K54 capsule was assessed by two separate methods that utilized various CP9 constructs with disruptions of rcsA, rcsB, Ion, rcsA and Ion and rcsB and Ion. The first method measured changes in activity of two independent group 2, K54 capsule gene protein fusions. The second method evaluated the serum sensitivity of these strains. These results established that RcsA serves as a negative regulator of group 2 capsule production in a functionally significant manner. RcsA's negative regulatory activity on the group 2, K54 capsule is greatest at 28°C and is RcsB dependent. This negative regulatory effect decreases with a rise in temperature. The existence of another Lon protease sensitive, negative regulator of the K54 capsular polysaccharide was also suggested. This project has been terminated at N.I.H.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00621-04 LCI
Project Terminated

PERIOD COVERED

October 1, 1993 - December 31, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Transformation by Epstein-Barr Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Jeffrey I. Cohen - Senior Staff Fellow, LCI, NIAID

COOPERATING UNITS *(if any)*

D. Mosier, Scripps Research Institute, La Jolla, CA

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epstein-Barr virus (EBV) nuclear protein 2 (EBNA-2) is essential for B cell transformation by the virus. The goal of this project is to determine the mechanism of B cell transformation by EBV.

Injection of EBV-transformed human lymphocytes into SCID mice results in the appearance of fatal human B cell tumors containing EBV. Cell lines were developed that contained EBV mutants that were identical, except for different deletions in their EBV EBNA-2 gene. EBV mutants with deletions in the amino half of EBNA-2 had reduced transforming activity in vitro and produced tumors in mice more slowly, while a carboxy-terminal mutant had a transforming activity greater than or equal to wild-type virus and produced tumors more rapidly than wild-type. Therefore, EBNA-2 is important both for lymphocyte transformation in cell culture and B cell tumor growth in SCID mice.

An EBV-transformed human B cell line, from one of the EBV mutants, was found to grow rapidly and widely disseminate in SCID mice. Analysis of these transformed cells isolated from mice showed a 1:18 chromosomal translocation and downregulation of the adhesion molecule, LFA-1A. These two abnormalities may be related to the increased tumorigenicity of this cell line.

EBV EBNA-2 contains a domain that directly activates transcription; a 14 amino acid region possesses much of the transcriptional activity and is essential for transformation by EBV. A chimeric virus was constructed that contained a portion of the transcriptional activation domain from the herpes simplex virus VP16 inserted in place of the 14 amino acid region in EBNA-2. This chimeric virus was able to transform B lymphocytes and transactivate expression of EBV and B cell genes. Mutation of a single amino acid in the 14 amino acid domain of EBNA-2 abolished transcriptional activation and B cell transformation. Thus, transcriptional activation is required for EBV-induced B cell transformation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00650-03 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Chronic Hepatitis B Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.E Straus - Senior Investigator, LCI, NIAID
R. Mc Kenzie - Medical Officer, LCI, NIAID

COOPERATING UNITS *(if any)*

J. Hoofnagle (NIDDK)
J. Stotka (Eli Lilly Co., Indianapolis, Indiana)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been exploring the potential antiviral efficacy of a class of fluoropyrimidine nucleoside analogues. Initially we explored the drug FIAC for inhibition of CMV infection in AIDS patients. Antiviral activity was not seen at tolerated doses and studies turned to the drug's metabolic derivative, FIAU. Studies showed intolerance at high doses but excellent tolerance and profound inhibitory activity at lower doses against hepatitis B virus (HBV). The initial studies involved HIV positive patients with co-existing chronic HBV infection. Profound and even permanent reductions in circulating HBV DNA and antigens were achieved with oral FIAU at daily doses of 0.1, -.5, or 1 mg/kg for 14 days. We then turned to a broader dose modification study in normal patients with chronic HBV infection. Studies compared antiviral efficacy at .05, 0.1, 0.25 and 0.5 mg/kg per day for 28 days. We noted profound inhibition of HBV infection even at the lowest doses and in the absence of any substantial side effects. By 9 months after treatment 9 of 24 patients had lost HBV DNA; 2 of which lost HBeAg. Based on these findings additional HIV-negative chronic HBV patients were enrolled in a randomized study comparing 0.1 and 0.25 mg/kg per day for 6 months to determine long term safety and efficacy. The trial was terminated 6/26/93 because of the emergence of life-threatening toxicity in the study. The past year of work entailed long term follow up of the study patients and a multi-faceted exploration of the cellular and biochemical bases for the toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI00651-03 LCI
Project Terminated

PERIOD COVERED

October 1, 1993 to July 28, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Recurrent Urinary Tract Infections In Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas A. Russo, M.D., C.M., Senior Staff Fellow, NIAID, LCI

COOPERATING UNITS *(if any)*

Walter Stamm, M.D., Seattle, WA.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Pathogenesis Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The urinary tract is one of the commonest sites for bacterial infection. Strains of E. coli are responsible for greater than 80-90% of these episodes. Urinary tract infections inyoung women account for an estimated 7 million office visits per annum and more thana billion dollars of direct medical costs. Further, 20% of women will develop frequent recurrences. Understanding the mechanisms through which recurrences occur anddevising preventative strategies is thus of considerable importance and is presentlypoorly understood. We utilized pulsed-field gel electrophoresis, to evaluate E. colistrains prospectively collected from 23 women with recurrent urinary tract infections. A subgroup of 6 women was studied to determine whether bacterial persistence within the urinary tract occurs or whether the colon is a reservoir for reinfecting strains.

We determined that: 1) Genotypes of isolates from a given individual with recurrent urinary tract infections are the same the majority of the time. 2)The majority of recurrences are due to bacterial reinfection, not persistence. 3)The faeces serves as the reservoir for re-infecting strains.

These results have significant therapuetic implications for women with recurrent UTI. This project has been terminated at N.I.H.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01A100654-03-LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics, Biochemistry and Therapy of Candida albicans.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

John E. Bennett, M.D. - Head, Clinical Mycology Section, LCI, NIAID
Others: Antonia Geber, M.D. - Guest Researcher

COOPERATING UNITS (if any)

Christopher A. Hitchcock, Ph.D. - Pfizer Central Research - Sandwich, England
Douglas Ward, M.D. - Washington, DC
Virginia Kan, M.D. - VAMC - Washington, DC

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH - Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several genes involved in sterol synthesis have been cloned from C. glabrata and C. albicans. The ERG3 gene, which encodes the 5,6 desaturase, has been cloned and sequenced from both species. The gene has been deleted from C. glabrata. The deletion has been confirmed by PRC, Southern and Northern analysis. The major sterol of this deletion mutant is fecosterol, which is consistent with a lack of desaturase activity. The deletion of this gene in C. glabrata does not, however, affect susceptibility to triazole antifungal agents. This differs from what has been observed in the closely related yeast S. cerevisiae and C. albicans, suggesting that the sterol requirement of C. glabrata differs from other related yeast. The C. glabrata ERG11 gene, encoding the cytochrome P-450-dependent x-14 demethylase, has been cloned and sequenced. Ura3 auxotrophic mutants in both the parent C. glabrata strain and it's erg3 deletion mutant were created by plating on 5-fluoro-orotic acid. Transformation with the S. cerevisiae URA3 gene revealed it to be functional in C. glabrata. A deletion construct using URA3 as a selectable marker has been made in order to disrupt this gene in C. glabrata and its' erg3 mutant. We have enrolled two patients in our clinical study: High Dose Fluconazole in the Treatment of Oropharyngeal Candidiasis Resistant to Low Dose Fluconazole. We have no data to report at this time.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01A100655-03-LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Pathogenic Fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute

John E. Bennett, M.D. - Head, Clinical Mycology section, LCI, NIAID

Others: Peter R. Williamson, M.D, Ph.D. - Medical Staff Fellow, Clinical Mycology Section, LCI, NIAID

Steven D. Salas, M.D. - Medical Staff Fellow, Clinical Mycology Section, LCI, NIAID

David Kruse, Ph.D. - Postdoctoral Fellow, Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH - Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Purified Cryptococcus neoformans diphenol oxidase was further characterized and found to be a laccase based on substrate specificity. Dopamine was shown by mass spectroscopy to be oxidized by the purified enzyme to an intermediate of melanin synthesis. The enzyme contained four moles of copper and showed absorbance spectrum consistent with type I and III copper binding sites. Trancriptional activity of the gene was derepressed in the absence of glucose, conditions which also increased enzyme activity. The gene CNLAC1 was expressed in Pichia pastoris, producing diphenol oxidase activity as a secreted protein. A melanin negative strain of C. neoformans was complemented by transformation with a DNA construct containing a genomic fragment of CNLAC1. The complemented strain produced melanin pigment and had diphenol oxidase activity. CNLAC1 was disrupted using a genomic fragment containing CNLAC1 interrupted by the URA5 gene. The disrupted strain had no detectable diphenol oxidase activity and produced no pigment. Biochemical and molecular methods are being used to identify and isolate the first two enzymes in capsule polysaccharide synthesis in C. neoformans. The GDP-mannose pyrophosphorylase is being cloned by using homology found in the prokaryotic enzyme as a basis for PCR primer construction. The PCR product was subcloned, sequenced and used to identify a cDNA clone which is being sequenced. The second enzyme, x-1,3 mannosyltransferase in being purified using standard biochemical techniques. The enzyme has been identified using the photolabeling radioactive substrate 8-azido-GDP-mannose and localized in acrylamide gels. The isolated enzyme band will be analyzed for amino acid sequence. This sequence will be used to construct degenerate oligonucleotide probes with which to isolate a cDNA clone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01A100657-03-LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on Candida and Apsergillus Species

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

K.J. Kwon-Chung, Ph.D. - Research Microbiologist - Clinical Mycology Section, LCI, NIAID
Others: Mark J. Parta, M.D. - Medical Staff Fellow - Clinical Mycology Section, LCI, NIAID
Yun C. Chang, Ph.D. - Senior Staff Fellow - Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

NIAID, NIH - Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although it is known that aspergillosis is caused by inhaled condidia, little is known about the precise events and interactions with the host that lead to successful survival and propagation of the fungus. During the initiation of aspergillosis, the surface of the inhaled conidia is the first fungal structure that comes in contact with the host. A. fumigatus condidia (spores) are known to be covered by a layer of hydrophobic proteinaceous fascicles called rodlets. The rodlet layer is believed to be responsible for the hydrophobic nature and the dispersibility of the spores. It has been shown that resting conidia of A. fumigatus with intact rodlet layers are killed much less effectively by human neutrophils than the swollen, rodletless hydrophobic conidia. It has been shown that hydrophilic conidia are more susceptible to neutrophil oxidative products and rabbit neutrophil cationic peptides. In addition, macrophages are also less able to kill hydrophobic spores than hydrophilic spores. We have cloned a gene responsible for the hydrophobicity of A. fumigatus conidia to study the survival of the conidia in the host tissue. PCR primers were constructed on the basis of highly conserved regions of the hydrophobin genes from three fungi were applied to A. fumigatus genomic DNA and a PCR product of 400bp fragment was isolated. The sequence of PCR product from A. fumigatus genome showed that it contained high sequence similarity with roda gene of Aspergillus nidulans. The PCR product was used for cloning the hydrophobin gene from genomic and cDNA libraries of A. fumigatus. A 4kb genomic DNA fragment and 1kb cDNA fragment were cloned and sequenced. The gene has two introns and codes a protein of 160 amino acids containing the 8 cysteines at the expected positions. The gene was designated as HYP1. HYP1 is developmentally regulated and expressed only when the culture reaches the sporulation stage. HYP1 gene was found to complement roda mutant of A. nidulans and restored rodlet layer in the fungus. This indicates that the HYP1 gene encosed the rodlet layer in A. fumigatus and that the gene is expressed in a heterologous host.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00674-02 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Varicella-Zoster Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.I. Cohen - Senior Staff Fellow, LCI, NIAID

T. Heineman - Medical Staff Fellow, LCI, NIAID

K.E. Seidel - Bio. Lab. Tech., LCI, NIAID

J. Ross - Medical Staff Fellow, LCI, NIAID

COOPERATING UNITS *(if any)*

D. Burton, Scripps Research Institute, La Jolla, CA

L. Stanberry, Children's Hospital, Cincinnati, OH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	2.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Varicella-zoster virus (VZV) causes chickenpox and herpes zoster. We have developed a system to generate recombinant VZV by transfecting tissue culture cells with four overlapping VZV cosmid DNAs. The recombinant VZV will be useful to study the role of individual VZV genes in infection.

Mutations have been engineered into cosmids that yield VZV mutants that are unable to express the viral thymidylate synthetase, glycoprotein V, ribonucleotide reductase, and ORF10 genes. Virus deleted for the ribonucleotide reductase gene grows at a slower rate than wild-type virus in vitro and is more sensitive to acyclovir than parental virus. While the HSV-1 homolog of ORF10, VP16, is essential for replication of HSV-1 in cell culture, deletion of ORF10 from VZV does not result in impaired growth in vitro. Selected VZV mutants will be used to inoculate guinea pigs to determine if the viruses have lost the ability to establish latency in the central nervous system.

The E. coli beta-galactosidase gene, has been inserted into the VZV genome, and the resultant virus produces plaques that stain blue with X gal. Guinea pigs are being inoculated with this virus to determine the types of cells that are able to support viral replication and establishment of latency. The herpes simplex virus (HSV) glycoprotein D (gD) gene, encoding a major neutralizing antigen, has been inserted into VZV and the resulting virus expresses high levels of HSV gD on the surface of infected cells. Inoculation of guinea pigs with this VZV mutant resulted in development of neutralizing antibodies to HSV-1. When the animals were challenged with live herpes simplex virus they showed reduced severity of herpes simplex infection compared with control animals.

Two combinatorial libraries of E. coli expressing human Fab fragments that recognize VZV have been generated and screened for neutralizing activity against VZV.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00680-02 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Novel Cytokines From Activated Macrophages

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joshua M. Farber, NIAID	Investigator	LCI/NIAID
Others:	Fang Liao	Visiting Fellow	LCI/NIAID
	Doron Amichay	Visiting Fellow	LCI/NIAID
	Hwang-Ho Lee	Visiting Fellow	LCI/NIAID
	Ronald Rabin	Clinical Associate	LCI/NIAID

COOPERATING UNITS *(if any)*

Philip Murphy, LHD, NIAID; Ronald Gress, EIB, NCI; Stephen Shaw, EIB, NCI; Giovanna Tosato, CBER, FDA; Padmavathy Vanguri, University of Maryland; Keith Peden, CBER, FDA; Robert Siliciano, Johns Hopkins University; F. Allen Sher, LPD, NIAID.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Cytokine Biology Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.1	4.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of the project is to identify and characterize macrophage products of potential importance in immune and inflammatory responses in order to manipulate these responses for clinical benefit.

Differential screening of a cDNA library prepared from a mouse macrophage-like cell line led to the identification of 11 mRNA species of increased abundance following exposure to gamma-interferon. Two of these mRNA species, designated Mig and crg-2 encode previously undescribed members of a newly-defined family of small secreted proteins, termed chemokines, that includes platelet factor 4, melanoma growth stimulatory activity (MGSA/gro) and IL-8 among others. Using the mouse MuMig cDNA probe, a new human member of the family has been discovered.

Work to date has demonstrated that the MuMig and HuMig mRNAs accumulate in monocytic cells specifically in response to gamma interferon, while Crg-2/IP-10 can respond to alpha interferon and to lipopolysaccharide as well. Following intraperitoneal injection of gamma interferon into mice, MuMig is induced in a range of tissues including most prominently liver, spleen, thymus, and uterus.

Chinese hamster ovary cell lines were derived that overexpress the MuMig, HuMig, Crg-2 and IP-10 proteins. Using a Western blot assay, the MuMig and HuMig proteins have been purified. By PAGE, the Mig proteins show multiple species with mobilities corresponding to 16-20 kDa for MuMig and 9.5-35 kDa for HuMig.

The size heterogeneity of the HuMig protein is due to proteolytic processing of the carboxy terminus. Functional studies have revealed that activated T cells, but not neutrophils or monocytes, are targets for HuMig.

Ongoing work is concentrating on determining the range of biological effects of Mig on T cells; on additional biochemical characterization of Mig and identification of Mig receptors; on the role of Mig in the response to infection; on a possible role for chemokines in embryogenesis; and on identifying additional novel chemokines.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00687-02 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Function Analysis of the Immediate-Early Protein IE62 of Varicella-Zoster Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI: L.P. Perera, DVM, Ph.D. - Senior Staff Fellow, LCI, NIAID
S.E. Straus, M.D. - Senior Investigator, LCI, NIAID

COOPERATING UNITS *(if any)*

G.S. Hayward, Ph.D., Johns Hopkins University, Baltimore, Md., J.D. Mosca, Ph.D., Henry M. Jackson Foundation, Rockville, MD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Varicella-zoster virus (VZV), a neurotropic alphaherpes virus, is the etiologic agent of two distinct clinical syndromes: chickenpox (varicella) and shingles (zoster). VZV is an enveloped virus with a linear double-stranded genome of approximately 125,000 base pairs (bp). The complete genomic nucleotide sequence of VZV has been determined and predicts approximately 70 unique genes. The expression of VZV genes appear to e temporally regulated and three putative kinetic classes have been defined for VZV genes; namely immediate early, early and late. Evolving evidence suggest that the putative immediate early gene product of ORF62 (IE62) to be the dominant regulatory protein in VZV. In addition, the ORF62 gene has been shown to be transcriptionally active during the latent phase of VZV infection in ganglia, thus suggesting a potential role for this gene in the maintenance of latency.

Current efforts focus on the structure-function analysis of IE62 regulatory protein using a variety of genetic approaches. The 75-amino acid activation domain of the IE62 is being probed to identify amino acids of high informational content, both by site-specific mutagenesis and regionally-directed, saturation mutagenesis. Our recent studies have illustrated that the IE62-dependent activation or responsive promoters are mediated by a unique mechanism involving the TATA element of the eukaryotic promoters. However, the magnitude of transcriptional activation appears to be imparted by a region of the protein other than the N-terminal activation domain. Consistent with this observation, we have identified a region that maps to the central portion of the 1310-amino acid polypeptide that physically interact with the two critical basal transcription factors, namely, TBP and TFIIB. These studies, thus have presented us with the challenge of dissecting the mechanism of transcriptional activation by a potent viral transactivation domain lacking any inherent ability to directly interact with either the TBP or TFIIB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00688-02 LCI

PERIOD COVERED

October 1, 993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Herpesvirus Interaction with Cell Surface Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard K. Williams, Ph.D. - Senior Staff Fellow - LCI, NIAID

S.E. Straus, M.D. - Senior Investigator - LCI, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms of binding and entry of varicella zoster virus (VZV) into cells are unknown. We are investigating the binding of VZV to cell membrane preparations from a variety of cultured cell-lines, to isolated proteins, glycoconjugates, and to synthetic peptides. Inhibition of VZV infectivity in diploid human lung cells in being used assess potential receptor-mimicking molecules for ability to block VZV binding and infection of cells. Monoclonal and polyclonal antibodies to cell membrane proteins are being developed to identify the specific cell surface molecules that serve as receptors for VZV. We have found that heparin inhibited infectivity of VZV in contrast another glycosaminoglycan chondroitin sulfate did not inhibit VZV infectivity. VZV binding to cell membrane preparations was measured. VZV binding was inhibited by added heparin suggesting that an important mechanism of interaction of VZV with cells involves heparin. Several assays were developed to measure binding of VZV and of herpesvirus glycoproteins to immobilized heparin. VZV was found to bind to immobilized heparin. Soluble VZV gB (gpII) has been produced using the vaccininia-T7 expression system to investigate glycoprotein to heparin receptor binding using ELISA based and biosensor technologies. This system will allow identification of glycoprotein structures critical to interaction between VZV and heparin receptors of cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00704-01 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cytokine production in animals infected with Histoplasma Capsulatum

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert Seder, M.D.	Investigator	LCI/NIAID
Others:	Ping Zhou, M.D.	Fogarty Fellow	LCI/NIAID

COOPERATING UNITS *(if any)*

Mycology Section, LCI

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Lymphokine Regulation Unit

INSTITUTE AND LOCATION

NIAID, National Institute of Allergy and Infectious Disease, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to understand cytokine regulation in animals infected with Histoplasma Capsulatum. Histoplasmosis is a disease found in certain geographic regions of this country. In a normal host the disease will often be controlled without significant end organ damage. However, patients infected with HIV have a difficulty in eradicating the disease. Therefore it was of interest to study mechanisms which might alter the immune response to this organism with the goal of protecting the animal.

Previous work had demonstrated that the organism resides in mononuclear phagocytic cells. Moreover, it was shown that the presence of IFNg enables the macrophage to kill the organism. Recently, a new cytokine, IL-12 has been shown to be a potent inducer of IFNg from both T cells and NK cells. Thus we set out to investigate the role that IL-12 had in the course of the disease and how it affected cytokine production especially IFNg.

We injected mice with a human isolate of Histoplasma Capsulatum, and then injected them with various cytokines at the onset of infection and followed them for mortality . We found that mice infected with 6x105 organisms died two weeks following infection. If mice were treated with an antibody against IFNg at the time of infection to neutralize endogenous IFNg, then death occurred in 7-10 days. Mice treated with IL-12 at the time of infection were still alive at two months. This data indicates that IFNg is critical for host protection and exogenous IL-12 can enhance survival. Further studies will be done to examine the cytokines made by the animal and determine whether mice receiving IL-12 had diminished number of organisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00705-01 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cytokine Production from Patients Infected with HIV and Tb.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert A. Seder, M.D., Lymphokine Regulation Unit, LCI, NIAID

Others: John McDyer, M.D., Medical Staff Fellow, LCI, NIAID

Daniel Lucey, M.D., Experimental Immunology Branch, NCI

Gene Shearer, Ph.D., Experimental Immunology, Branch, NCI

COOPERATING UNITS *(if any)*

Experimental Immunology Branch, NCI

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Lymphokine Regulation Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human HIV infection is a disease which evolves over a long period of time with a progressive loss of CD4+ T cells. The loss of CD4 T cell function both qualitatively and quantitatively diminishes the capacity of the host to respond to various infectious diseases. The ability to prevent the gradual loss of CD4+ T cells has been difficult to achieve to this point. However, there has been substantial interest in studying the qualitative function of CD4+ T cells as the course of disease progresses. Some studies using PBMCs have suggested that a switch in lymphokine production occurs from Th 1 to Th 2 over the course of disease and this precludes a more rapid clinical deterioration. Moreover, it has been suggested that responses to antigen can be restored in vitro by culturing cells in the presence of IL-12. The following studies will examine the regulation of lymphokine production and the ability to proliferate to defined antigens from both PBMC and purified CD4+ T cells at various stages of the disease. These studies will try to confirm earlier work provide new insights as to how the CD4+ T cell is regulated and whether its qualitative function can be altered.

In a small number of experiments it appears that patients infected with HIV are unable to produce IL-4 from PBMC or CD4+ T cells in response to various mitogens or antigens. Moreover, both PBMC and CD4+ T cells were unable to respond to a panel of conserved HIV peptide antigens and this response was not altered by the addition of IL-12. Studies will be continued on a large number of patients, focusing on establishing the type of lymphokine phenotype from both PBMC's and purified CD4+ T cells. Moreover, newer cytokines will be studied to see if they affect the qualitative and quantitative function of these aforementioned cell types.

Recently there has been a resurgence in the number of patients infected with Mycobacterium Tuberculosis in both HIV+ and HIV - populations. Moreover, the development of multi-drug resistant Tuberculosis has developed into a major health problem. Using the same techniques as outlined above, we will examine the functional capabilities of PBMC's and CD4+ T cells from patients infected with Tuberculosis. Understanding cytokine regulation in vitro and determining whether any functional defect exists may provide a rationale for in vivo

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00708-01 LCI

PERIOD COVERED

October 1, 1993 to Setember 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Clinical Basis of Food Allergy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D. Head, Allergic Diseases Section, LCI/NIAID

COOPERATING UNITS *(if any)*

Indian Institute of Science, Bangalore, India (P.V. Subba Rao)
Clinical Neuroscience Branch, NIMH (Martin)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.20	0.10	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major shrimp allergen has been isolated and identified as a tropomyocin based on a comparison of amino acid sequences of different peptides generated by proteolysis of the parent molecule. Dot blot and competitive ELISA inhibition using sera of shrimp sensitive patients revealed that antigenic as well as allergenic activities were associated with two peptides. These peptides have been sequenced. These two peptides are similar between cross-reacting Crustaceae, but vary between tropomyosin from phylogenetically distinct species. Synthetic peptides corresponding to two IgE binding B-cell epitopes have comparable allergenicity to native peptides.

Peripheral blood mononuclear cells from patients with allergic eosinophilic gastroenteritis (AEG) over-produced IL-4 and IL-5 with mitogenic stimulation. IL-5 synthesis by $CD4^+$ T lymphocytes occurred in the absence of mitogen.

Gamma interferon mRNA was not detected by reverse transcription/polymerase chain reaction in gut mucosal biopsies from patients with AEG, but was present in controls.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00709-01 LCI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lymphokine Profiles in Asthma and Allergic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Dean D. Metcalfe, M.D., Head, Allergic Diseases Section, LCI/NIAID
Others: Calman Prussin, M.D., Medical Staff Fellow, LCI/NIAID

COOPERATING UNITS *(if any)*

Laboratory of Parasitic Diseases (Dr. Thomas Nutman)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.10	1.10	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using three color flow cytometric analysis for the detection of intracellular cytokines, we have studied peripheral blood T cell subpopulations to determine the cytokines produced by individual lymphocytes. Cells from normal individuals and helminth infected patients were magnetically sorted for the CD4+CD27+ and CD4+CD27- subpopulations. Intracellular staining for IL-4, IL-5, and IFN-γ, subsequent to mitogen stimulation revealed that the CD4+CD27- population included higher frequencies of cells capable of producing IL-5 and not IFN-γ, IL-4 not IFN-γ and IFN-γ and neither IL-4 nor IL-5 compared to the CD4+CD27+ population. Thus, while virtually no CD4+CD27- lymphocytes produce both IL-5 and IFN-γ, a distinct proportion produced both IL-4 and IFN-γ (0.1-8.0%) and 66-84% of IL-5-producing cells also produce IL-4. Lymphocytes from normals and patients had the same functional T cell subsets, but the CD4+CD27- lymphocytes from patients had higher frequencies of cells producing IL-4 or IL-5. Normal subjects had higher frequencies of cells producing IFN-γ. This data demonstrates Th1, Th2, and Th0 cell subsets within the CD4+CD27- lymphocyte population. Studies are underway to examine both peripheral blood and broncho-alveolar lavage-derived lymphocyte populations in allergic individuals with asthma.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00710-01 LCI (New)

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Homologs of Herpesvirus Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.I. Cohen - Senior Staff Fellow, LCI, NIAID

K.E. Seidel - Biol. Lab. Tech., LCI, NIAID

COOPERATING UNITS *(if any)*

M. Spriggs, Immunex, Seattle, Washington

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Herpesviruses cause latent infections that persist for the lifetime of the host and these viruses have developed mechanisms to counteract host defenses so as to allow the virus to persist. Using sophisticated computer programs we are identifying herpesvirus genes that have cellular homologs. These genes are being expressed in various systems to determine how the genes may interact with host cell proteins, including those of the immune system, to influence the course of infection. Identification of these genes may help define new targets for antiviral therapy or new insights into modulating the immune system.

Varicella-zoster virus (VZV) ORF13 encodes a viral thymidylate synthetase. This enzyme catalyzes the methylation of deoxyuridylate to thymidylate. We have constructed a VZV mutant that cannot express the viral thymidylate synthetase by inserting stop codons into the gene. We plan to inoculate guinea pigs with the VZV mutant to determine whether the viral thymidylate synthetase is important for infection with the virus or for its ability to spread to the central nervous system and maintain latent infection.

Epstein-Barr virus (EBV) encodes a protein that contains seven putative transmembrane domains and shares homology to the G protein-coupled receptor superfamily. We have cloned this EBV gene, expressed a protein, and are currently assaying the protein for biological activity.

Additional herpesvirus homologs of known mammalian proteins have been identified. Promising candidates will be studied in appropriate systems in collaboration with M. Spriggs at Immunex.

LABORATORY OF HOST DEFENSES
1994 Annual Report
Table of Contents

ZO1 AI Project Number		Page
Summary		6-1
00155-19	Clinical Studies of Abnormal Host Defense - Gallin	6-6
00481-09	Human Phagocyte NADPH Oxidase - Rotrosen	6-7
00521-07	Effect of Cytokines in Host Defense and Inflammation - Gallin	6-8
00614-04	Structure and Function of Phagocyte Proteins - Leto	6-9
00615-04	Molecular Analysis of Neutrophil Activation by Chemoattractants - Murphy	6-10
00644-03	Biology of Peripheral Blood Progenitors: A Target for Gene Transfer - Malech	6-11
00645-03	Gene Therapy for Immune Deficiencies - Malech	6-12
00646-03	Transgenic Animals Models of Human Immune Defects - Holland	6-13
00647-03	Genes and Gene Products as Immuno-Adjuvants - Holland	6-14
00648-04	NADPH Oxidase Genes (Inactive) - Leto	6-15
00649-03	Human Phagocyte Granule Proteins - Rosenberg	6-16

PHS-NIH

Summary Statement

LABORATORY OF HOST DEFENSES

National Institute of Allergy and Infectious Diseases

October 1, 1993 to September 30, 1994

Introduction

The objective of the LHD is to integrate basic and clinical research of the role of phagocytic cells in host defense and inflammation. The focus of work is (1) to study the physiology, biochemistry and molecular biology of phagocytic cells (blood neutrophils, mononuclear phagocytes, eosinophils) in host defense against bacteria and fungi; (2) to determine the role and effect of cytokines on both phagocytic cells and upon the general process of inflammation; (3) to investigate the cellular, biochemical and genetic basis of diseases leading to recurrent bacterial infections, particularly diseases affecting phagocytic cell function (chronic granulomatous diseases of childhood (CGD), Hyperimmunoglobulin E-recurrent infection syndrome, neutrophil specific granule deficiency, leukocyte adhesion deficiency, cyclic neutropenia and others); (4) to develop new animal models of human diseases which lead to recurrent infections; (5) to develop new treatments for host defense defects leading to recurrent infections including new antibiotic or cytokine therapy or prophylaxis against infection; and (6) to develop gene therapy for the inherited defects of phagocyte function. In addition to these studies the LHD serves as a national resource for assisting physicians throughout the country (and the world) in the evaluation of patients with host defense defects.

During the past year the Laboratory has continued and expanded upon clinical studies in patient cohorts collected at NIH over more than 20 years. Studies have been performed in patients and normal volunteers regarding the physiology and biochemistry of phagocytic cells and cytokines in infection and inflammation. The oxidative microbicidal superoxide-generating NADPH oxidase, the microbicidal granule proteins, and the chemoattractant receptors of phagocytic cells were studied.

While detailed descriptions of individual projects are included in the individual project reports, the following is a list of some of the major accomplishments of the laboratory:

o Four-year follow up of CGD patients receiving interferon γ reveals continued efficacy and no serious adverse side effects.

o Demonstration that mononuclear cells from patients with disseminated infection with multiple drug-resistant (MDR) atypical mycobacterial have diminished production of interferon-γ.

o Demonstration that interferon-γ has important therapeutic potential for disseminated infection with MDR atypical mycobacterial infection and description of a family with probable X-linked predisposition to atypical mycobacterial infection.

o Characterization of the orderly recruitment of cytokines into skin blisters of humans. Demonstration that exudate neutrophils are induced to synthesize and release IL-8.

o Characterization of the appearance of cytokines and demonstration of elevated E-selectin in the circulation following endotoxin administration to normal human volunteers.

o In vitro correction of stem cells from patients with each of the four types of chronic granulomatous disease (CGD) using gene transfer technology.

o Creation of mouse embroyonal stem cell knock-outs for p47phox as a precursor for a CGD mouse deficient in p47phox.

o Demonstration that src homology 3 (SH3) domains are required for the assembly of NADPH oxidase components. Demonstration of proline rich sequences as targets for SH3 binding during NADPH oxidase assembly; a mutation of one proline in p22phox accounts for one of the CGD genotypes.

o Demonstration that the cytochrome b component of NADPH oxidase contains NADPH and FAD binding domains.

o Cloning and sequencing of human and mouse clusters of cross-hybridizing genes that encode heptahelical, rhodopsin-like, G-protein coupled phagocyte chemoattractant receptors or related products of unknown function; sequence relationships indicate these genes arose from a common ancestor.

Clinical Studies

A LHD study completed four years ago demonstrated the efficacy of prophylactic interferon-gamma to reduce infection frequency in patients with chronic granulomatous disease (CGD). We have entered the fourth year of a phase IV study aimed at determining the long term safety for the patients and the continued effect of interferon-γ on both phagocytic cells and the inflammatory process. This treatment appears to be without adverse effects and patients continue to benefit from interferon-γ. A double-blinded randomized trial was continued to determine whether prophylactic oral itraconazole can reduce fungal infections in CGD. In related studies on the patient group with CGD, data were collated and published relating to infections with unusual fungal organisms.

Studies of patients with Mycobacterium avium intracellulare (MAI) have been expanded. A family group with X-linked susceptibility to MAI was identified, with a possible lymphocyte defect. A protocol is in progress which is examining the use of interferon-γ to enhance host defense against MAI in this patient group. To date, twelve patients have been studied. These studies have been very promising with a majority of patients showing dramatic benefit from the interferon-γ therapy.

Studies of the use of interferon-γ altering the humoral and cellular defects seen in hyperige-recurrent infection syndrome (HIE) are also in progress. This and other studies of use of interferon-γ in treatment of MAI and CGD noted above will yield important information about the use of this cytokine in various syndromes in which patients are prone to infection.

Clinical studies of normal volunteers and patients were continued to determine the systemic and local changes in cytokines and phagocytic cells in response to both local inflammation, modelled by experimental skin blisters and systemic responses modeled by reaction to intravenous bacterial endotoxin. The time course for the accumulation of several cytokines and other

mediators was documented. C5a, LTB4 IL1 and IL-8 appeared within 3 h in blisters; IL-6 G-CSF, and TNF-α appeared from 8-12 h. Following IV endotoxin TNF-α, IL-8, IL-6 and IL-r antagonist appeared within 4 h while G-CSF appeared after 8 h. Of particular note was an early and dramatic increase in E-selectin following increasing doses of endotoxin, suggesting this may be useful parameter to monitor in patients suffering from endotoxemia. Patients with HIE had a ten-fold normal increase in tumor necrosis factor in local lesions yet showed normal systemic responses to endotoxin. High levels of tumor necrosis factor in pulmonary tissues of HIE patients during staphylococcal pneumonia might cause the extensive tissue damage to lungs seen following infection in this disease. In related studies, administration of endotoxin to normal volunteers was associated with four-fold increases in circulating hematopoietic progenitor cells. This may reflect an important role for circulating progenitors in bacterial infections. Furthermore, endotoxin itself or the cytokines increased by endotoxin, such as IL-6 or G-CSF may be useful agents to enhance the yield of progenitor cells harvested from patients for gene therapy approaches to treatment as discussed below.

A clinical study was initiated to look at the recruitment of CD34 peripheral blood progenitors from the marrow in response to administration of G-CSF. Early results of this study indicate that an optimum dose is 5 ug/kg/day administered subcutaneously for 5 or 6 days. In most individuals this results in an increase in circulating CD34 cells from 1000 cells/ml whole blood to over 40,000 CD34 cells/ml whole blood. The harvest of progenitors by apheresis can yield as many as 150 million CD34 cells after purification. These results are important to the planning for a future gene therapy protocol for chronic granulomatous disease.

In another study G-CSF was used to increase the apheresis harvest of granulocytes from normal donors and these cells used in a study of administration to CGD patients. Early results provide data about the kinetics of consumption of granulocytes administered to CGD patients.

Studies of the development of new treatments

Studies have been initiated to develop methods of gene transfer of normal genes for the phagocyte NADPH oxidase into hematopoietic progenitor cells of CGD patients defective in such genes. These studies are aimed at gene therapy for CGD. Defective retroviruses free of helper virus activity were developed which were capable of transferring expression of normal oxidase genes in CGD cells defective in such genes. In a manuscript published in the past year the correction of the most common X-linked form of CGD was demonstrated and follows up on similar studies published from LHD with the p47phox deficient autosomal recessive form of CGD. Studies are in progress now to scale up these results to allow the initiation of a clinical protocol testing the safety of gene transfer methodology for treatment of CGD. These studies involve production of replication incompetant retrovirus under GMP conditions and the development of culture conditions for efficient transduction of large numbers of progenitor cells from CGD patients.

Basic science studies of phagocytic cells

Studies of the growth of peripheral blood progenitor cells in culture are essential to development of gene therapies based on the introduction of new genes into these cells. Conditions have been defined to optimized growth toward the neutrophil, monocyte or eosinophil lineage. Specifically, the combination of stem cell factor, Interleukins-3 and 6, GM-CSF, and G-CSF is optimal for neutrophil differentiation and SCF, IL-3 and GM-CSF for monocyte/eosinophil production. The combination of IL-3, GM-CSF and IL-5 result in almost exclusively eosinophil development from CD34 cell hematopoietic progenitors.

Recently, in our laboratory all of the protein components of the phagocyte superoxide-generating NADPH oxidase have been identified. The cytosolic factors include the previously identified p47phox and p67phox, while the third reburied cytosol factor was shown to be the low molecular weight GTPase, rac2. In related studies, the only membrane components of the oxidase was shown to be cytochrome b558 (a p22phox/gp91phox heteromer) which is a heme and flavin containing NADPH binding protein. During the past year studies have been conducted which have defined critical binding domains in the different oxidase subunits which interact. Specifically the src-homology 3 domains (SH3) of the p47phox and p67phox proteins appear to bind to target proline rich domains of the p22phox and the p47phox respectively. In related studies the SH3 domains were shown to be absolutely essential for oxidase activation in the intact cell, but may not be essential in a cell-free activation of the oxidase, suggesting the importance of these domains for the complex events occuring in the intact cell. In other studies, functional aspects of the structure of the flavocytochrome b558 were delineated. Functional domains were identified which were important for the oxidase activation. Studies indicate that the stoichiometry of subunit ratio may be two of the p22 subunits to one of the gp91 subunits.

Considerable progress has occurred toward the goal of a CGD knockout mouse model. Specifically, founder and F1 generation mice with a knockout of the p47phox CGD gene have been produced and homozygous mice with the CGD defect should be available soon. This will allow animal modelling of granuloma formation, the treatment of infections in CGD, and the testing of gene therapy systems.

Studies of the expression of eosinophil granule proteins as recombinant proteins in E.coli have allowed the definition of sequence elements in these proteins which target the binding of a foldase (chaparone protein) groEL. This work was published during this year. A chaparone-like protein hsp70RY, has been identified in EBV-transformed B-lymphocytes and characterized. An understanding of chaparone proteins will be important for delineating the production and targeting of phagocyte granule proteins of all kinds. In other studies of the eosinophil granule proteins EDN and ECP, the ribonuclease activity and the bactericidal activities have been separated. These studies may yield information about protein structure motifs that are important to antimicrobial activities.

We have previously established the 1° structure, expression and signaling properties of the human N-formyl peptide receptor (FPR), the FPRL1 receptor (FPRL= formyl peptide receptor like), interleukin-8 receptor type B (IL8RB), and the MIP-1α/RANTES receptor from cDNA and gene cloning. We have also previously defined the complete DNA sequence and structural organization of the human FPR gene including an analysis of its putative promoter sequence, and have proposed a mechanism for the molecular evolution of the FPR gene family. We have now cloned the gene and cDNA for the putative FPRL2 receptor which is 56% identical in amino acid sequence to FPR and 83% identical to FPRL1R, yet does not bind prototype N-formylpeptides such as N-formyl-met-leu-phe (fMLF). Unlike FPR and FPRL1R, whose RNAs are detectable in both normal human neutrophils and monocytes, the FPRL2 mRNA is detectable in monocytes but not neutrophils. We have also cloned the gene for the murine homologue of FPR and characterized its product as a low affinity receptor for fMLF. Analysis of chimeric FPR:FPRL1R receptors indicated that the high affinity binding site for fMLF is formed by the apposition of multiple non-contiguous extracellular domains. These data and sequence comparisons among the high and low affinity fMLF receptors have pointed to five residues that may be particularly important for high affinity ligand binding and signal transduction. We discovered that open reading frame *ECRF3* of *Herpesvirus saimiri* encodes a protein with 33% amino acid sequence identity to IL8RB that is a functional receptor for the same ligands that activate IL8RB (IL-8, GRO/MGSA and NAP-2). The MIP-1α/RANTES receptor also has a

viral homologue, *US28* of human cytomegalovirus. These findings contribute to an emerging theme in DNA virology whereby molecular mimicry of host defense proteins is accomplished by gene copying. We have previously shown that proteins involved in host defense functions are highly and exceptionally divergent between human and rodent species, and have proposed a new hypothesis to account for this phenomenon that has to do with molecular mimicry by acquisition of host genes by species-specific pathogens.

Administrative, Organization, and Other Changes

Dr. John I. Gallin continued as the Chief and Dr. Harry L. Malech's title was changed from Assistant to Deputy Chief ofthe Laboratory. Dr. Murphy continues as a tenure scientist. Dr. Thomas Leto will be proposed for tenure in the coming year. Dr. Helene Rosenberg and Dr. Steve Holland continue as tenure track scientists in the Laboratory.

Honors, Awards, and Scientific Recognition

Dr. John I. Gallin was on May 1, 1994 appointed Director of the Clinical Center, NIH and Associate Director of Clinical Research for NIH. In addition Dr. Gallin was in 1993 asked to serve as Special Consultant to the President and Dean, The Mount Sinai Medical Center, New York, New York. In addition he was appointed to the Scientific Advisory Committee for the Immune Deficiency Foundation's Chronic Granulomatous Disease Registry. Dr. Gallin served as President of the International Society for the Immunocompromised Host and this year organized the international meeting of this society in Davos, Switzerland. He continues to serve on the editorial board of numerous journals and was invited to serve as Visiting Professor and to give numerous plenary lectures at major meetings in FY93.

Dr. Harry L. Malech was in FY94 appointed to the Editorial Board of the publication Immune Deficiency and Allied Disorders: Clinical Updates. He continues as an Ad Hoc Reviewer for the US Food and Drug Administration. He was recently appointed a member of the NIH Patent Policy Board, Subcommittee on Cooperative Research and Development Agreements. He was also appointed to the Georgetown University Department of Medicine Chairman's Advisory Committee. Dr. Malech was an invited lecturer at many national and international conferences in the United States, Japan and Europe and is sought as a consultant by academic and industrial institutions.

Dr. Philip M. Murphy in FY94 was given the NIH Director's Award for his work on chemokines, was appointed to the editorial board of the Journal of Leukocyte Biology, and was invited to lecture and consult at many academic and industrial institutions.

Dr. Thomas Leto in FY94 was a lecturer and session chairperson at the European Society for Clinical Research in Toledo, Spain, and has been an invited to speak and consult for academic and industrial institutions.

Dr. Steven M. Holland in FY94 received an NIAID award for his work on development of a mouse model of CGD. He also served as a chairperson for a session on cytokine therapy in the meeting of the International Society for the Immunocompromised Host in Davos, Switzerland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-19 LHD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies of Abnormal Host Defense

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:	Harry L. Malech, M.D.	Deputy Laboratory Chief	LHD/NIAID
	Steven M. Holland, M.D.	Senior Staff Fellow	LHD/NIAID
	Ellen DeCarlo	Research Nurse	LHD/NIAID
	Judi Miller	Research Nurse	LHD/NIAID

COOPERATING UNITS *(if any)*

D Kuhns, Ph.D., PRI-Frederick Cancer Research Facility; Julia Lekstrom, JE Bennett, & KJ Kwon-Chung, LCI/NIAID; HI Pass, Surgery Branch/NCI; M Sneller, LIR/NIAID; Genentech

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study patients with abnormal host defense. In FY '94 studies focussed on patients with abnormal phagocyte function. These include patients with chronic granulomatous disease of childhood (CGD), Hyperimmunoglobulin E-recurrent infection sydrome (Job's), specific granule deficiency, leukocyte adhesion deficiency and other patients with recurrent infections who do not fall into a specifically defined disease category. Currently we follow 77 patients with CGD, 18 patients with the hyperimmunoglobulin-E recurrent infection syndrome, and 18 patients with other phagocyte dysfunction syndromes. In addition, 12 patients are followed with disseminated multiple drug resistant-atypical mycobacteria infections, some of whom appear to have abnormalities of phagocytic cells. A phase IV study of the effect of interferon-γ for infection prophylaxis in CGD has continued at NIH at the specific request of the FDA. No unexpected toxicities have been seen with interferon-γ. The reduction in infections observed in the initial study has been sustained and of note is that the incidence of fungal infections in CGD patients receiving interferon-γ is half that for those patients not receiving the drug. In view of the high incidence of fungal infections in CGD patients, we have also initiated a study designed to assess the efficacy of itraconazole in CGD. Several long term studies were completed related to specific management issues of CGD patients. These included comprehensive reviews of skeletal involvement in CGD, special anesthetic considerations in CGD, unique urologic manifestations in CGD, the surgical management of pulmonary infections in CGD, and the surgical pathology of the lung in CGD. Each of these clinical studies could only be compiled at NIH where a large cohort of CGD patients could be followed for a long time. Several unique infections and their successful management were described in CGD. These include reports of the successful treatment of Sarcinosporon inkin, Exophiala dermatitidis, and Paecilomyces varioti. The encouraging findings in CGD patients prompted consideration of the use of interferon-γ in other infectious diseases.As part of our long term objective of evaluating interferon-γ in tuberculosis, we initiated studies of its use in an unusual patient population with disseminated atypical mycobacteria infection resistant to conventional therapy. Twelve patients have been studied to date. Dramatic clinical benefit with eradication of the infection was seen in the patients treated.

S 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00481-09 LHD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Phagocyte NADPH Oxidase

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Daniel Rotrosen, M.D.	Medical Officer	LHD/NIAID
Others: Choh L. Yeung	Biologist	LHD/NIAID
Harry L. Malech, M.D.	Asst. Chief, LHD	LHD/NIAID

COOPERATING UNITS *(if any)*

Yoichi Osawa, Ph.D., LCP, NHLBI
John Coligan, Ph.D., BRB, NIAID

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Laboratory of Host Defenses

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project explores the structure and biochemistry of the phagocyte NADPH oxidase. A basic understanding of the biochemistry of this electron transport chain is critical to development of novel agents to modulate the inflammatory response.

The focus is on the structure of the phagocyte-specific cytochrome b558 which we showed is a complex flavocytochrome comprised of two subunit proteins, gp91phox and p22phox. Despite knowledge of the primary structure of the cytochrome subunits, little is understood about their biosynthesis and assembly. To begin to characterize these events we have used metabolic inhibitors of heme biosynthesis, heme deprivation, and protein and immunochemical approaches to explore regulation of cytochrome biosynthesis and processing in native and recombinant systems. Findings lay the experimental foundation for a new paradigm currently under investigation in which heme transporters or chaperone-like molecules involved in heme insertion play a regulatory role in biosynthesis of heme-containing enzymes.

Studies in progress address the stoichiometry of gp91phox and p22phox subunits in the cytochrome. Both physicochemical crosslinking and recombinant engineering suggest that the cytochrome is a heterotrimer comprised of two heme-bearing p22phox subunits, and a single flavin-bearing gp91phox. Findings challenge the previously accepted view of the cytochrome as a simple gp91phox-p22phox heterodimer and provide fresh insights into the search for the cytochrome heme binding domains.

Oligopeptides which mimic probable contact sites between gp91phox and p47phox are being developed as candidate inhibitors of oxidase assembly.

S 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00521-07 LHD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Cytokines in Host Defense and Inflammation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
Others:	Ellen DeCarlo	Research Nurse	LHD/NIAID

COOPERATING UNITS *(if any)*

Douglas Kuhns, Ph.D., PRI-Frederick Cancer Research Facility

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NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies were performed to define the accumulation of cytokines and other mediators of inflammation in experimental models of inflammation in normal human subjects and in patients with abnormalities of host defenses or inflammation. Skin blisters created by suction and heat were used to study local inflammation in the skin. The kinetics of the appearance of inflammatory cytokines in the blister fluid revealed mediators appearing early (C5a, LTB4, IL1β, and IL-8) and mediators appearing late at 12 hrs or later (TNF-α and GM-CSF) with IL-1α and IL-2 not detected. Studies of skin blisters in patients with abnormal inflammatory responses revealed dramatic increases in TNF-α in patients with acute vasculitis in association with Wegeners granulomatosis, in patients with systemic mastocytosis, and in the syndrome of hyperimmunoglobulin E and recurrent infections. Increases in TNF-α correlated well with disease activity suggesting that targeting drug development for modulation of this cytokine will have specific merit in these disease states. Normal blister exudate neutrophils synthesize large amounts of IL-6, IL-8, and TNF-α compared with control peripheral blood neutrophils. Studies of normals and patients with excessive inflammation revealed a tight linear relationship between the level of IL-8 and the number of inflammatory neutrophils in the exudate. Exudate neutrophils synthesize IL-8 which is stored in a compartment different from specific or azurophil granules, but is co-eluted with an alkaline phosphatase rich plasma membrane fraction. E. coli endotoxin was administered to normal volunteers and patients in accordance with a long standing NIAID protocol. Increased plasma levels of TNF-α, TNF-α receptor, IL-1α receptor antagonist, IL-6 and IL-8 occurred within 4h and G-CSF increased by 6 h, but not changes in IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-10, interferon-γ, TGF-β or C5a were seen. There was a striking increase in circulating E-selectin by 2-4 h after endotoxin administration which was dose related suggesting that E-selectin may be predictive of those patients with endotoxemia who will progress to shock. Definition of specific mediators that are abnormal in different disease states is important for developing therapies. In other studies the long term monitoring of patients with chronic granulomatous disease of childhood who are receiving interferon gamma as infectious prophylaxis indicated no adverse effects. In other studies interferon-γ was shown to be useful in enhancing host defense against infection with mycobacteria including MAI.

S 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00614-04

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Phagocyte Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Thomas L. Leto, Ph.D.	Senior Staff Fellow	LHD/NIAID
Others:	Isabelle Ossona de Mendez, Ph.D.	Visiting Fellow	LHD/NIAID
	Cheung H. Kwong, M.D., Ph.D.	Special Volunteer	LHD/NIAID
	Anthony G. Adams, B.A.	Biologist	LHD/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Host Defenses

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2		

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☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project explores structure-function relationships in important host defense systems of phagocytic cells. Current efforts are focused on the assembly of the NADPH oxidase, an enzyme responsible for production of superoxide anion and related microbicidal oxidants. We have recently proposed a model for NADPH oxidase assembly involving interaction of conserved Src Homology 3 (SH3) domains in two cytosolic components (p47-phox and p67-phox) with proline-rich target sequences in other oxidase components. The model is based on in vitro binding experiments, using isolated domains of oxidase components, and oxidase assembly studies that exploit recently developed gene transfer technology. We have shown that a point mutation in one of the putative proline-rich targets in the cytochrome b558 small subunit (p22-phox, P156Q) that has been associated with chronic granulomatous disease (CGD) interferes with membrane translocation of p47-phox and disrupts p47-phox SH3 binding. The model proposing direct p47-p22-phox interactions was further supported by inhibitory effects of two point mutations in the first SH3 domain of p47-phox and experiments showing that expression of p22-phox alone is sufficient to enable p47-phox translocation. Similar experiments involving mutagenesis of a second proline-rich target sequence (p47-phox, res. 358-372) confirmed the importance of yet another SH3-mediated interaction, observed between the C-terminal domains of p67-phox and p47-phox. We have also recognized another structural motif, identified by computer searches as 34 residue repeats within p67-phox. Their role is being explored with regards to oxidase reconstitution and binding to another essential oxidase component, Rac. Structural features critical to oxidase regulation by this Ras-related GTPase have been defined through analysis of chimeric Rac proteins that contain sequences from inactive Ras homologs. These studies may provide a structural basis for design of agents that can modulate this important aspect of the inflammation and may facilitate developments in the area of gene therapy for CGD. The work also provides insights on a variety of other signal transduction systems that involve structurally related proteins or protein sequence motifs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00615-02 LHD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Neutrophil Activation by Chemoattractants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Philip M. Murphy, M. D.	Senior Investigator	LHD/NIAID
Others:	Ji-Liang Gao, Ph. D.	Visiting Associate	LHD/NIAID
	Sunil Ahuja, M. D.	Visiting Associate	LHD/NIAID
	H. Lee Tiffany, M. S.	Biologist	LHD/NIAID
	Christophe Combadiere, Ph. D.	Visiting Scientist	LHD/NIAID

COOPERATING UNITS *(if any)*

Christine Kozak, Ph. D., NIAID/LMM
Elmer Becker, M. D., Ph. D., University of Connecticut Health Center
Richard Freer, Ph. D., Medical College of Virginia

LAB/BRANCH

Laboratory of Host Defenses

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously established the 1° structure, expression and signaling properties of the human N-formyl peptide receptor (FPR), the FPRL1 receptor (FPRL= formyl peptide receptor like), interleukin-8 receptor type B (IL8RB), and the MIP-1α/RANTES receptor from cDNA and gene cloning. We have also previously defined the complete DNA sequence and structural organization of the human FPR gene including an analysis of its putative promoter sequence, and have proposed a mechanism for the molecular evolution of the FPR gene family. We have now cloned the gene and cDNA for the putative FPRL2 receptor which is 56% identical in amino acid sequence to FPR and 83% identical to FPRL1R, yet does not bind prototype N-formylpeptides such as N-formyl-met-leu-phe (fMLF). Unlike FPR and FPRL1R, whose RNAs are detectable in both normal human neutrophils and monocytes, the FPRL2 mRNA is detectable in monocytes but not neutrophils. We have also cloned the gene for the murine homologue of FPR and characterized its product as a low affinity receptor for fMLF. Analysis of chimeric FPR:FPRL1R receptors indicated that the high affinity binding site for fMLF is formed by the apposition of multiple non-contiguous extracellular domains. These data and sequence comparisons among the high and low affinity fMLF receptors have pointed to five residues that may be particularly important for high affinity ligand binding and signal transduction. We discovered that open reading frame *ECRF3 of Herpesvirus saimiri* encodes a protein with 33% amino acid sequence identity to IL8RB that is a functional receptor for the same ligands that activate IL8RB (IL-8, GRO/MGSA and NAP-2). The MIP-1α/RANTES receptor also has a viral homologue, *US28 of human cytomegalovirus*. These findings contribute to an emerging theme in DNA virology whereby molecular mimicry of host defense proteins is accomplished by gene copying. We have previously shown that proteins involved in host defense functions are highly and exceptionally divergent between human and rodent species, and have proposed a new hypothesis to account for this phenomenon that has to do with molecular mimicry by acquisition of host genes by species-specific pathogens.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00644-03

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of peripheral blood progenitors: a target for gene transfer.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
Others:			
	Michael Mardiney, MD,	Clinical Associate	LHD/NIAID
	Steven Rafferty, PhD,	Visiting Fellow	LHD/NIAID
	Joseph Domechowski, MD,	Special Volunteer	LHD/NIAID
	Fei Li, Ph.D.	Visiting Fellow	LHD/NIAID
	Meir Shalit, M.D.	Clinical Associate	LHD/NIAID
	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID

COOPERATING UNITS *(if any)*

Sudhir Sekhsaria, MD IS/CP/CC; Thomas Fleisher, MD IS/CP/CC;
Susan Leitman, MD BSS/DTM/CC

LAB/BRANCH

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies peripheral blood hematopoietic progenitors (PBHP) as a target for gene therapy of inherited diseases affecting the function of human phagocytic cells, including neutrophils, eosinophils, and monocytes. A related goal of this project is development of novel cellular therapies based on genetically engineering human PBHP and their phagocytic cell progeny to endow them with new properties to augment host defense against chronic infections including tuberculosis and other mycobacteria infections. To achieve these goals it is necessary to define conditions for optimal harvest, purification and culture of the primitive human hematopoietic cells with a CD34 surface antigen phenotype. In a clinical study we determined that 5 ug/kg daily subcutaneous granulocyte colony stimulating factor administered for 5 or 6 daily doses is optimal for recruiting a 20 to 100 fold increase in circulating primitive CD34 progenitor cells. Using apheresis and magnetic bead purification technology over 100 million purified CD34 cells can be harvested from G-CSF recruited volunteers. These cells can be used as a target for gene transfer studies. The recruited cells are more receptive to retrovirus mediated gene transfer than similar CD34 cells from volunteers not treated with G-CSF. In related studies we find that the combination of the growth factors SCF, IL3 and IL6 used to culture these cells preserves the CD34+ primitive phenotype longest in culture. We developed specific culture conditions leading to predominantly neutrophils, eosinophils or monocyte/macrophages. This will be important for future studies aimed at engineering new characteristics into specific end stage phagocytes by gene transfer into progenitors. As part of our studies to achieve gene therapy for the inherited immunodeficiency, chronic granulomatous disease (CGD), a sensitive FACS assay of superoxide/hydrogen peroxide production by mature neutrophils was developed based on the oxidation induced increase in fluorescence of rhodamine-123. Using this assay it was possible to follow the maturation of functional oxidase as a marker of the appearance and number of maturing neutrophils over time in cultures of PBHP. This information is essential for planning and evaluating studies in which genes are transferred into CGD progenitors to correct NADPH oxidase activity as gene therapy for CGD.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00645-03

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene therapy for immune deficiencies.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Harry L. Malech, M.D.	Assistant Laboratory Chief	LHD/NIAID
Others:			
	John I. Gallin, M.D.	Laboratory Chief	LHD/NIAID
	Michael Mardiney, MD,	Clinical Associate	LHD/NIAID
	Steven Rafferty, PhD,	Visiting Fellow	LHD/NIAID
	Joseph Domechowski, MD,	Special Volunteer	LHD/NIAID
	Fei Li, Ph.D.	Visiting Fellow	LHD/NIAID
	Robert Sokolic	Guest Researcher	LHD/NIAID

COOPERATING UNITS *(if any)*

Sudhir Sekhsaria, MD IS/CP/CC; Thomas Fleisher, MD IS/CP/CC; Michael Gottesman, LCB/DCBDC/NCI; Susan Leitman, MD BSS/DTM/CC; Warren Strober, LCI, NIAID;

LAB/BRANCH

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NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies gene therapy for chronic granulomatous diseases (CGD) and other inherited immune diseases affecting human phagocytes, and also studies use of gene therapy to augment phagocyte host defense against chronic intracellular infections such as tuberculosis and other mycobacterial infections. CGD are a group of 4 distinct genetic disorders with common phenotype characterized by life-threatening recurrent infections caused by failure of blood neutrophils and monocytes to produce superoxide and hydrogen peroxide. CGD results from the failure to produce any of the components of the NADPH oxidase. We have achieved retrovirus mediated gene transfer to functionally correct 3 genetic forms of CGD by transfer of normal oxidase genes into cultured hematopoietic progenitor cells. The target cells are marrow-like myeloid progenitor cells which can be harvested from the peripheral blood resulting in 1% to 20% correction of superoxide production by phagocytes differentiated from CGD progenitors transduced with normal oxidase gene. Work is in progress to build on these accomplishments to develop a clinical protocol of gene therapy for CGD. Several host defense genes have been cloned which may play a role in host resistance to intracellular pathogens responsible for certain indolent infections such as tuberculosis. Nitric oxide synthase producing nitric oxide in murine macrophages appears to be important for control of intracellular pathogens such as tuberculosis, though this host defense mechanism is not firmly established in human phagocytes. We have achieved constituitive expression of murine macrophage NOS gene in human macrophage cells lines with resultant constant production of nitric oxide. This will be an important model to test the hypothesis of the potential host defense utility of nitric oxide production by human phagocytes.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00646-03

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transgenic Animal Models of Human Immune Defects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	Steven M. Holland, MD	Senior Staff Fellow	LHD/NIAID
Others:	Sharon H. Jackson, MD	Clinical Associate	LHD/NIAID
	David M. Frucht, MD	Research Associate	LHD/NIAID
	John I. Gallin, MD	Laboratory Chief	LHD/NIAID
	Eli Eisenstein, MD	Clinical Associate	LCI/NIAID
	Warren Strober, MD	Section Chief	LCI/NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at understanding the role of specific enzyme systems in generating and maintaining host immunity. The approach taken involves the creation of mice with discrete defects in host defenses by using homologous recombination of DNA into the host genome to disrupt relevant genes. Lesions will be introduced that either correspond to previously identified mutations in humans or that incapacitate systems probably involved in host defense. Therefore, this project relies upon a thorough understanding of the molecular and functional organization of the NADPH oxidase system and the mutations which disable it. Further, the dissection of specific phenotypes associated with defined genotypes may provide insight into the importance of certain functional domains of the genes of the NADPH oxidase and indicate which sites are most informative for further study.

We have used the NADPH oxidase system as a paradigm for the creation of mice with a genetic defect similar to one found in humans, chronic granulomatous disease (CGD). To this end, we have cloned, characterized and disrupted genomic fragments from both the mouse $p47^{phox}$ and gp91phox genes. Disruption of these genes has provided the basis for a murine model of the autosomal recessive and X-linked forms of CGD, respectively. Embryonal stem cells with homologous recombination at the p47phox gene have been generated and successfully microinjected into mouse blastocysts. Chimeric fertile males have been generated which have successfully sired heterozygous knock-out pups. These animals are being bred to homozygosity. The disrupted gp91phox gene has been electroporated into embryonal stem cells and screening for the homologous recombinant clones is underway.

In the course of this work we have 1) further characterized a family with ineffective granulomatous inflammation as demonstrated by disseminated *Mycobacterium avium* infection, 2) overcome the inflammatory defect in the family with disseminated *Mycobacterium avium* infection by treatment with interferon gamma, 3) identified immune defects in patients with this infection.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00647 03

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genes and gene products as immuno-adjuvants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven M. Holland, MD	Senior Staff Fellow	LHD/NIAID
Others:	Sharon H. Jackson, MD	Clinical Associate	LHD/NIAID
	Choh Yeung, BA	Biologist	LHD/NIAID
	David M. Frucht, MD	Research Associate	LHD/NIAID
	John I. Gallin, MD	Laboratory Chief	LHD/NIAID

COOPERATING UNITS *(if any)*

Thomas C. Quinn, MD	Senior Investigator	LIR/NIAID

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at understanding, characterizing, and evaluating a novel form of gene product delivery for immunization and treatment: direct DNA injection. Cytokines will be administered intramuscularly to mice in the form of either a cDNA expression vector or preformed protein simultaneously or in sequence with cDNAs for specific antigens from chlamydia or other infectious agent in order to elicit a strong immune response. Animals will be followed by evaluation of cytokine response in the circulation, local response to the injected materials at the site of administration, and development and type of immunity. To these ends we have obtained and/or engineered into expressible forms the cDNAs for mouse interferon gamma, interferon alpha$_1$, TNF-alpha, IL-2, IL-4, and IL-6.

The development of a mouse model of chronic granulomatous disease provides an important system in which to evaluate the possible therapeutic role of intramuscular interferon gamma cDNA injection. The recognition and preliminary characterization of a spontaneous animal model of disseminated *M. avium* complex infection in the Matschie's tree kangaroo, has led to the effort to clone the marsupial interferon gamma gene. The cloning of this gene will provide the source for the cDNA to be used in experiments of immune augmentation in this species.

In order to develop a source for antigens needed for these studies, we have generated DNA libraries in a bacterial plasmid system from both *Chlamydia trachomatis* and *Chlamydia pneumoniae*. From these libraries we have cloned the genes for the chlamydial major outer membrane proteins and the chlamydial 57kd heat shock protein. These genes have been transferred into bacterial and eukaryotic expression systems.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00648-04

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

NADPH Oxidase Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Thomas L. Leto, Ph.D. Senior Staff Fellow LHD/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Building 10, Rm 11N 106, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00649-03-LHD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Phagocyte Granule Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Helene F. Rosenberg, M.D., Ph.D.	Investigator	LHD/NIAID
	Kimberly D. Dyer, M.S.	Biologist, GS-9	LHD/NIAID
	Malathi Sathyamoorthy, Ph.D.	Post-doctoral Fellow	LHD/NIAID

COOPERATING UNITS *(if any)*

Laboratory of Intracelluar Parasites, RML, NIAID (R.P. Morrison), Graduate Genetics Department, George Washington University (H. Lee Tiffany)

LAB/BRANCH

Laboratory of Host Defenses

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseasess, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to elucidate the molecular events involved in the biosynthesis and activation of eosinophil and neutrophil granule proteins.

A. Eosinophilopoiesis. We have shown that the disorder known as neutrophil specific granule deficiency (SGD) also includes the eosinophil lineage (Rosenberg HF and Gallin JI. Blood 82:268-273 (1993)), as three of the four granule proteins are absent in circulating eosinophils from an affected individual. We have gone on to show that circulating CD34+ progenitor cells isolated from normal individuals can be induced to differentiate toward eosinophils that have transcriptional defects mimicking SGD (Rosenberg HF et al (1994) In review). We have also identified a cell line in which transcription of these deficient granule proteins can be studied directly (Tiffany HL and Rosenberg HF (1994) In review).

B. Granule protein folding. We have described the high affinity, reversible interaction of the prokaryotic molecular chaperone, groEL, with nascent chains of two eosinophil granule proteins (Rosenberg HF et al, J. Biol. Chem. 268:4499-4501 (1993)). We are developing a eukaryotic recombinant system in which folding of these proteins can be studied in their native locale. Also, using a panel of monoclonal antibodies, we have identified a novel protein that cross-reacts with a specific anti-chaperone antibody (Rosenberg HF et al (1994) In review). We have also further characterized another molecular chaperone protein (hsp70RY) which was initially identified in EBV-transformed B cells (Dyer KD and Rosenberg HF. Bioch. Biophys. Res. Commun. (1994) In press.)

C. Structure and activity of ECP and EDN. Eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) are both ribonucleases, and have structural homology to other members of the ribonuclease gene family. Using a unique approach, we have succeeded in expressing recombinant ECP and EDN in a prokaryotic system, and have shown that, for ECP, antibacterial activity is unrelated to ribonuclease activity (Rosenberg HF. 1994. In review). In addition, using antibodies that distinguish between storage and secreted forms of ECP, we have results suggesting that the released form is deglycosylated relative to the storage form (Rosenberg HF and Tiffany HL. J. Leuk. Biol. (1994) In press.)

S 6040 (Rev. 5/92)

LABORATORY OF IMMUNOGENETICS

1994 ANNUAL REPORT

TABLE OF CONTENTS

Z01-AI Project Number		Page
Summary		10-1
00166-17	Characterization of Rabbit MHC Antigens -- Kindt	10-9
00168-17	Cell Surface Markers of Rabbit Lymphocytes -- Kindt	10-10
00170-17	Molecular and Functional Analysis of Human Class II Histocompatibility Antigens -- Long	10-11
00171-17	Infection of Rabbits with Human Immunodeficiency Virus 1 -- Kindt	10-12
00180-16	Properties of Transformed Rabbit Cell Lines -- Kindt	10-13
00389-10	Molecular Genotype Analyses of HLA and TCR Genes in Humans -- Robinson	10-14
00525-07	Molecular Analysis of Human Killer T cells -- Long	10-15

LABORATORY OF IMMUNOGENETICS

National Institute of Allergy and Infectious Diseases
October 1, 1993 to September 30, 1994

RESEARCH PROGRESS

Studies in the Laboratory of Immunogenetics emphasize genes involved in immune function and molecular mechanisms involved in responses to immunization and infection. Molecular dissection of antigen processing has shed new light on the cellular events crucial to mounting an immune response and suggest a novel pathway by which class II MHC molecules may process endogenous antigens. Studies on natural killer cells include cloning of a novel cell surface molecule involved in directing their cytolytic activity.

Genetic mapping studies of the human TCR gene complex describe sequences for genetically determined regions of insertion/deletion; analyses of these sequences show that this area encodes functional genes. Studies of TCR gene expression reveal restricted usage of V_{beta} genes in response to hepatitis B antigen immunization. Experimental infection of rabbits with the human retrovirus, HTLV-I suggests mechanisms by which HTLV-I causes disease in certain individuals but not in others. Molecular clones of the virus have been obtained and will be used to investigate specific gene function. In studies of HIV-1 infection in rabbits, animals transgenic for human CD4 have been obtained and are being tested for ability to support HIV-1 infection.

Molecular Mechanisms of Antigen Presentation

These studies aim to define the function of human class II molecules (HLA-DR) in their interaction with T cells and the requirements for class II-restricted processing and presentation of viral antigens to CD4-positive T cells. Antigen processing for presentation by class II molecules generally involves endocytosis of antigen into an acidic compartment through which newly synthesized class II molecules travel on their way to the cell surface. The invariant chain with which class II molecules associate immediately after biosynthesis is responsible for the transport of class II molecules to a compartment within the cell where antigen processing takes place. An intracellular class II-like molecule, termed HLA-DM, is necessary for efficient antigen presentation. Once at the cell surface, the foreign peptide/MHC class II complex interacts with the T cell receptor and the CD4 molecule expressed on the antigen-specific T cell. It was demonstrated here that a new processing pathway can be used for the presentation of exogenous antigen to CD4 T cells. This new pathway is independent of newly synthesized class II molecules transported to processing compartments by the invariant chain, and independent of HLA-DM. In contrast, it involves mature cell surface class II molecules that internalize by endocytosis and recycle to the cell surface. This new pathway may be very useful for the presentation of antigens that are rapidly degraded upon uptake into antigen processing cells. In addition, an endogenous

processing pathway leads to the presentation of cytosolic antigen to class II-restricted T cells. It was shown here that this pathway is distinct from the class I antigen presentation pathway and that it requires HLA-DM. Thus, it appears that both forms of antigen, exogenous and endogenous, are targeted to the same intracellular compartment for processing. This previously unsuspected presentation of endogenous proteins by class II molecules has important implications for selection of the T cell repertoire, for T cell tolerance, and for autoimmunity. The intracellular transport of MHC class II molecules is a crucial step in successful antigen presentation. The invariant chain assembles with newly synthesized class II molecules and targets them to processing compartments located in the endocytic pathway. After dissociation of the invariant chain, class II molecules acquire immunogenic peptides and are transported to the cell surface where they can be sampled by T cells. The precise transport routes to and from antigen processing compartments are unknown. It was shown here that a significant fraction of newly synthesized class II molecules are transported to the cell surface before their delivery to processing compartments. The cytoplasmic tail of the invariant chain possesses a powerful endocytosis signal that mediates internalization of these surface class II-invariant chain complexes. Furthermore, it was demonstrated that transport of class II molecules from the processing compartments back to the cell surface follows a novel pathway. Proteins that are retrieved from late endocytic compartments to the cell surface are transported through the trans-Golgi network. In contrast, retrieval of intracellular class II molecules does not involve traffic through the trans-Golgi network, implying that a distinct transport route to the cell surface exists in antigen presenting cells. **(Roche, Pinet, Guéguen, Rojo, Warmerdam).**

NK Cell Specificity

Natural Killer (NK) cells are important effectors in the clearance of cells infected by viruses and by intracellular parasites. One of the fascinating aspects of NK cells is that their specificity in target cell recognition does not lie at the level of target cell molecules that trigger NK cells but, rather, is mediated by target cell structures that provide a dominant negative signal to NK cells which protects the target cell from NK cell lysis. This was first demonstrated with allospecific NK cell clones. HLA-B and HLA-C alleles expressed on target cells provide protection from lysis by NK cells. Each NK cell clone displays a particular specificity for a given set of HLA class I alleles. The aim of this study is to determine what form of HLA class I molecules is involved in the recognition by NK cells, and to isolate molecular clones encoding the receptor of NK cells that recognizes HLA class I molecules. By use of transfected cells expressing HLA class I molecules, as well as mutated forms of the HLA-B27 allele, it was shown here that NK cells can distinguish between different conformations of a given class I molecule. It is likely that the peptides bound to class I molecules affect the recognition by NK cells. An experimental system is being developed to test this possibility. The family of p58 receptors on NK cells is involved in providing the dominant negative signal upon interaction of the NK cell with a target cell expressing the appropriate HLA-C allele. To obtain molecular clones for this family of receptors, a p58 receptor was

purified from a NK cell line and subjected to amino acid sequence analysis. The amino-terminal sequence was obtained, as well as the sequence of several internal peptides obtained by digestion with trypsin. Based on these sequences it was possible to obtain molecular DNA clones corresponding to the p58 receptor. Interestingly, this receptor belongs to the immunoglobulin superfamily. Isolation of multiple cDNA clones has already led to the identification of molecules related to the p58 receptor family. These molecular probes provide a powerful tool to analyze NK cells with distinct functional specificities, to describe the extent of diversity in the p58 receptor family, to define how this diversity is genetically encoded and maintained between NK clones, and to explore the molecular mechanisms by which NK cells recognize target cells **(Malnati, Wagtmann)**.

Studies of the Human T-cell Receptor Complex

Genes encoded within the major histocompatibility complex (MHC) and T cell antigen receptor (TCR) gene complexes have been shown to play important roles in immune responses and in susceptibility to autoimmune diseases. The contribution of individual gene products within these gene complexes and the mechanisms by which they function have not been elucidated. In the present studies, inheritance patterns of HLA and T cell receptor (TCRA and TCRB) genes were analyzed in families. Structural analyses have led to a refined estimate of the extent of the germline TCRBV repertoire and to the identification of polymorphic gene segments and regions. Two frequently occurring insertion / deletion related polymorphisms (IDRP) were found in the TCRB gene complex; one involves a stretch of 21.5kb in the V region and another spans ~20kb near the C region. Three TCRBV genes are encoded in the inserted TCRBV region; BV7S3, BV9S2(P), and a second copy of BV13S2 which is identical to a gene present in all haplotypes. The inserted region shows a pattern of sequence identity with other regions of the gene complex that suggests genetic mechanisms for its origin may include unequal crossing over, gene conversion like events, insertion and deletion of short segments of DNA and mutation. Deleted haplotypes contain 60 genes of which 50 are functional, whereas, inserted haplotypes contained 63 gene of which 52 are functional. **(Zhao, Robinson)** Three BV genes were found to have null or unexpressed alleles. **(Barron, Deulofeut, Currier)** The utilized TCR repertoire was analyzed in specific immune responses. The TCR present on T cell lines with defined specificities were characterized and the extent of junctional diversity associated with each TCRBV family was determined. Oligoclonal populations of T cells expressing a few BV families were present on T cells responding to Hepatitis surface antigen. The BV genes used by different responder individuals were similar. **(Deulofeut)** Activated cells from patients with autoimmune diseases were analyzed in a similar fashion. Patients with Kawasaki's disease showed oligoclonal expansion of several BV families. **(Barron)** Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states.

Studies of the Rabbit MHC

Investigations of the major histocompatibility complex of the rabbit (RLA) have provided further evidence concerning the similarities and differences between RLA and the well characterized MHC regions of human and mouse. Investigations of the major histocompatibility complex of the rabbit (RLA) have led to identification of a nonpolymorphic gene encoding the class II DO beta chain. DOB has an overall structural similarity to other class II beta chains but unlike these, it is expressed only on mature B cells and to a limited extent in thymus. The function of the DOB chain is of interest because of its differences to other class II molecules. Our studies will involve expression of the gene in an E. coli protein system in order to prepare monoclonal antibodies against it **(Samaan, Mahana).** The gene encoding the putative alpha chain partner of DOB is being sought using probes for the human counterpart (DNAlpha) and candidate clones are being studied. Efforts to construct a complete physical map of the rabbit MHC are in progress and attempts to order the various MHC regions in the rabbit continue. Previous results showed that class II and class I regions were adjacent with no interposition of Class III genes. Studies of class III gene prompted a study of heat shock protein 70 which is expressed on HTLV-I infected cells. Members of this MHC linked protein family appear to play a role in immunity to pathologic consequences of retroviral infection (**Chouchane**).

HTLV-Infection in Rabbits

Human T cell lymphotropic virus-I (HTLV-I) is a retrovirus which is the etiologic agent of human adult T cell leukemia and a progressive neurologic disorder termed Tsp/Ham. Although these and various other human disease conditions have been associated with HTLV-I infection, the majority of HTLV-I infected individuals remain asymptomatic. Although hypotheses concerning the progression of HTLV-I infection to disease have been advanced, no clear explanation for the myriad activities of this virus is available. Structural studies of HTLV-I reveal a highly conserved sequence for isolates taken from diverse populations and from individuals with different disease manifestations. As opposed to HIV and SIV sequences which vary enormously, HTLV-I sequences seldom diverge more than several percent. No infectious molecular clones of HTLV-I have been reported, therefore it is not yet possible to relate biologic activity to the observed structural variation.

The rabbit can be readily infected with HTLV-I, the rabbit infection closely mimics that in humans in that HTLV-I only causes disease in certain instances. A series of HTLV-I cell lines were prepared by in vitro transformation of rabbit peripheral blood mononuclear cells (PBMC) using HTLV-I cell lines of human or rabbit origin as virus source (**Sawasdikosol**). The majority of HTLV-I lines obtained, as well as the human or rabbit progenitors of these lines, mediated asymptomatic infection in injected animals. Seroconversion and chronic cell infection were the only consequences even in rabbits injected with as many as 5×10^8 live HTLV-I infected cells. By contrast, certain infected lines killed rabbits in a dose dependent manner; animals injected with the lethal lines developed high

temperature within four days of injection and usually died by day 8. A detailed description of the disease shows similarity to adult T cell leukemia lymphoma in humans. Injection of irradiated cells was not lethal within the same short time frame as live cells, but most hallmarks of the disease were present (**Simpson**). The majority of rabbit HTLV-I cell lines including several derived in the same fashion as the lethal line cause no overt disease. A detailed study of this phenomenon is underway; results here can shed light on why HTLV-I infection in humans causes serious disease in only a small percentage of infected individuals whereas most infected persons remain asymptomatic. Efforts center about comparing lethal and nonlethal cell lines and the virus produced by them. Structure of virus from lethal and non lethal cell lines have been completed and molecular clones derived; some of these pass infection and can be used to dissect functional genes of the virus (**Zhao, Robinson**). Investigations of organs from rabbits infected with the lethal line showed apoptosis in the thymus and in the spleen; the non lethal virus caused no similar effect. In vitro studies further showed that virus purified from the lethal HTLV-I cell line causes apoptosis in rabbit or human PBMC, whereas that from a non lethal line causes cell proliferation (**Leno**). The targets of the virus are T cells.
Studies of antibodies to members of the heat shock protein families revealed an unexpected and potentially important correlation between anti hsp and survival to challenge with HTLV-I. Anti-heat shock protein (hsp) antibodies were observed in the sera of rabbits after inoculation with the HTLV-I cell lines. One cell line, RH/K34, which failed to raise a response to hsp70, causes lethal leukemia when live cells are injected into unrelated outbred rabbits. Injection of sublethal doses of this line failed to elicit anti hsp although injection of cell free virus from the line did so. Rabbits with detectable levels of anti hsp70 antibodies prior to challenge with lethal doses of live RH/K34 cells were resistant to its lethal effects suggesting that hsp immunity (which is autoimmunity) may influence the outcome of RH/K34 pathogenicity and may have general implications to viral immunity.

Infection of Rabbits with HIV-1

Infection in rabbits injected with HIV-1 has been detected by seroconversion, detection of virus in cells and organs using PCR and *in situ* hybridization, and by isolation of virus from PBMC. While the fact that rabbits can be infected with HIV is promising, the slow course of infection, difficulty in isolation of virus and the absence of consistent disease signs in infected rabbits have limited utility of this potential model for AIDS. Information gained in recent efforts aim to improve the model suggest that infection in the rabbit may be enhanced in several ways. Adaptation of HIV to grow more efficiently in rabbits has been attempted by long term in vivo passage; injection of as little as 1 ml of blood from animals receiving splenocytes from a rabbit infected with HIV-1 and held for 2.3 years has resulted in lymphopenia, CD4 T cell depletion and for 3 of 12 rabbits, death (**Simpson**).
The question of whether the slow progression of infection in rabbits is rooted in problems with transcription of viral DNA has prompted preparation of CAT constructs utilizing the 5' or 3' LTRS from HIV-1 to provide the promoter or 3' processing signals (**Cho**). These are being used to compare LTR-mediated gene

expression in rabbit and human cells. There are no apparent defects in promoter or processing of genes flanked by HIV-1 LTR's. In subsequent experiments, molecular clones of HIV-1 were used in transfection experiments to assess production of infectious virus in rabbit as compared to human cell lines (**Hague**). Both species produced virus that appeared normal by all criteria, including ability to infect human cells. When transfection efficiencies were comparable, lines from the two species produced equal quantities of virus. The measurement of infectivity on human target lines indicated that transfected rabbit cells produced a higher ratio of defective particles.

Rabbit CD4 and HIV Infectivity

Studies of cell surface markers for rabbit lymphoid cell populations have emphasized rabbit T cells with special reference to those involved in retrovirus infection. The rabbit CD4 (RbCD4) gene was used in transfection experiments to compare its efficacy as a receptor for HIV-I to human CD4 which is known to bind the env protein of HIV with high affinity. RbCD4 transfectants of human and mouse cell lines were compared with these lines expressing human CD4. RbCD4 proved to be a poor receptor for HIV-1. Because rabbit CD4 is not appropriate as receptor for HIV, rabbits transgenic for human CD4 were developed. Transgenic animals were shown to express HuCD4 on appropriate T lymphocyte populations and in a development specific fashion. Infections of PBMC from the transgenics with HIV-1 are more productive than normal rabbit infection (**Hague**). Preliminary data indicate that PBMC from the transgenic rabbits undergo apoptosis in the presence of cell free HIV-I whereas no effect on normal rabbit PBMC is noted (**Leno**). Several transgenic rabbit lines are being bred for study.

Rabbit Lymphoid Cell Markers

Other rabbit lymphoid cell marker investigated include the T cell γδ receptors, CD8ß and IL-2rα. Transcripts of the delta TCR gene occurred in two distinct forms, a duplication of exon 2 of the constant region was found in the larger message (**Sawasdikosol, Hague**). This was shown to result from a genetic polymorphism in which the gene encoding TCR Cdelta was duplicated along with some flanking intron sequences. This insertion deletion polymorphism (IDRP) was shown to be inherited as an autosomal codominant trait and animals homozygous for the inserted form had levels of γδ T cells in their peripheral blood that were equal to rabbits homozygous for the shorter form. Present studies continue to characterize T cell surface markers that may play a role in pathogenic effects of HTLV-I infected cells. Collaborative efforts are aimed at characterization of surface markers involved in other cell processes including adhesion. Antibodies for the products of the CD18 and CD11c genes are available from Dr. JM Wilkinson and efforts to clone the genes are underway.

LABORATORY OF IMMUNOGENETICS

ANNUAL REPORT

October 1, 1993 to September 30, 1994

HONORS AND AWARDS

Dr. Thomas Kindt, Chief of the Laboratory of Immunogenetics, presented invited seminars at the Pasteur Institut in Paris, at Transgene Inc in Strasbourg, at the Max Planck Institute for Immunobiology in Freiburg, he presented a seminar in the NIH wide immunology series, was guest lecturer at Amherst College and a plenary speaker at the Conrad Biochemistry Symposium in Urbana Illinois. Dr. Kindt was an invited participant at the symposium celebrating the 100th anniversary of the Institute Pasteur in Tunis, Tunisia. Laboratory data were presented at the meeting of the Laboratory of Tumor Cell Biology, and at the HTLV-I meeting in New Jersey. Dr. Kindt serves on the publication committee of the American Association of Immunologists and is an Associate Editor of the *FASEB Journal* and North American regional editor of *Research in Immunology*. Dr. Kindt served on the Board of Scientific Visitors for the Oklahoma Medical Research Foundation in Oklahoma City in November and, in June, completed 4 years of service on the NIH Allergy and Immunology Study Section. Dr. Kindt continues to serve on the scientific advisory boards of Oncor Inc., Gaithersburg MD, and Innovir Laboratories Inc., New York, NY.

Dr. Eric Long head of the molecular immunology section of LIG was an invited speaker in the Distinguished Speaker Series, Immunology Colloquium, University of Pennsylvania, and in the Immunology Seminar Series of the Scripps Research Institute, La Jolla, CA. Dr. Long was an invited participant to the 2nd Annual International Workshop on Natural Killer Cells, Taormina, Italy and served on the Board of Scientific Review for a Program Grant at Harvard Medical School.

Dr. Mary Ann Robinson, acting head of the Human Immunogenetics Research Section of LIG was an invited session chairman at the T cell Receptors and Disease meeting sponsored by the New York Academy of Science. She was an invited speaker at FASEB tutorial on T cell receptors and at the annual Inhibitors of Coagulation meeting. Dr. Robinson continues to serve on the Scientific Affairs Committee for the American Society of Histocompatibility and Immunogenetics (ASHI), was chairman of the Abstract Review committee and chaired a session at the ASHI 1993 annual meeting. She continues to serve as a member of the editorial board of Human Immunology. Dr. Robinson received the NIH Director's Award for her outstanding research efforts on human T cell genetics..

LABORATORY OF IMMUNOGENETICS

ANNUAL REPORT

October 1, 1993 to September 30, 1994

ADMINISTRATIVE REPORT

The Laboratory of Immunogenetics occupies laboratory and animal space in the NIAID Twinbrook II facility in Rockville, Maryland. Office space in the building was renovated and occupied in March 1994, completing the cycle of renovation for LIG space. The laboratory continues to maintain a contract facility at Spring Valley Laboratories for animals infected with human retroviruses.

The unit headed by Dr. Mary Ann Robinson, previously incorporated in the immunogenetics research section was given a separate budget this year and proposed as a section called "Human Immunogenetics Research Section"; the promotion of Dr. Robinson to section head status has been submitted and is under review.

In the past year the Immunogenetics Research Section of the laboratory was joined by Dr. Jeffrey Currier from Queensland University Australia will work with Dr. Mary Ann Robinson and by Drs. Angela Samaan and Wahib Mahana from University of Tours France who will work with Dr. Kindt. Departures include Dr. Lotfi Chouchane who will assume a faculty position in the medical school in Monastir, Tunisia, Dr. Sansana Sawasdikosol who joined the Dana Farber Institute in Boston and Dr. Paul Roche who has a tenure track position in the NCI. Special volunteers in the laboratory were Dr. Anne Simonsen from University of Oslo, Norway, Ms. Jeanette Blackford from the Royal College of Surgeons, London and Dr. Marta Peruzzi from University of Florence, Italy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00166-17

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Rabbit MHC Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.J. Kindt	Chief	LIG, NIAID
Other:	L. Chouchane	Visiting Fellow	LIG, NIAID
	A. Samaan	Visiting Fellow	LIG, NIAID
	W. Mahana	Visiting Fellow	LIG, NIAID

COOPERATING UNITS *(if any)*

Dr. Patrice Marche, Institut Pasteur, Paris

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations of the major histocompatibility complex of the rabbit (RLA) have led to identification of a nonpolymorphic gene encoding the class II DO beta chain. DOB has an overall structural similarity to other class II beta chains but unlike these, it is expressed only on mature B cells and to a limited extent in thymus. The function of the DOB chain is is of interest so the gene is being expressed in an E. coli protein system in order to prepare monoclonal antibodies against it. The gene encoding the putative alpha chain partner of DOB was sought using probes for the human counterpart and candidate clones are being studied. Efforts to construct a complete physical map of the rabbit MHC are in progress and attempts to order the various MHC regions in the rabbit continue. Previous results showed that class II and class I regions were adjacent with no interposition of Class III genes. Studies of class III gene prompted a study of heat shock protein 70 which is expressed on HTLV-I infected cells. Anti-heat shock protein (hsp) antibodies were observed in the sera of rabbits after inoculation with the HTLV-I cell lines. One cell line RH/K34, which failed to raise a response to hsp70, was shown subsequently to cause lethal leukemia when live cells were injected into unrelated outbred rabbits. Injection of sublethal doses of this line failed to elicit anti hsp although injection of cell free virus from the line did so. Rabbits with detectable levels of anti hsp70 antibodies prior to challenge with lethal doses of live RH/K34 cells were resistant to its lethal effects suggesting that hsp immunity (which is autoimmunity) may influence the outcome of RH/K34 pathogenicity and may have general implications to viral immunity.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00168-17

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Other:	Bishop F. Hague	Staff Fellow	LIG, NIAID
	Sansana Sawasdikosol	Biologist	LIG, NIAID
	R. Mark Simpson	Expert	LIG, NIAID

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Dr. J. Michael Wilkinson, Jeannette Blackford, The Wellcome Trust, London

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Laboratory of Immunogenetics

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National Institute of Allergy and Infectious Diseases, Rockville, MD 20852

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of cell surface markers for rabbit lymphoid cell populations have emphasized rabbit T cells with special reference to those involved in retrovirus infection. The rabbit CD4 (RbCD4) gene was used in transfection experiments to compare its efficacy as a receptor for HIV-I to human CD4 which is known to bind the env protein of HIV with high affinity. RbCD4 transfectants of human and mouse cell lines were compared with these lines expressing human CD4. RbCD4 proved to be a poor receptor for HIV-1 and therefore rabbits transgenic for human CD4 were developed. Transgenic animals have been obtained and shown to express HuCD4 on appropriate T lymphocyte populations and in a development specific fashion. Infections of PBMC from the transgenics with HIV-1 are more productive than normal rabbit infection. Preliminary data indicate that PBMC from the transgenic rabbits undergo apoptosis in the presence of cell free HIV-I whereas no effect on normal rabbit PBMC is noted. Several transgenic rabbit lines are being bred for study. Other rabbit lymphoid cell marker investigated include the T cell receptors $\gamma\delta$, CD8β and IL-2rα. Transcripts of the delta TCR gene occurred in two distinct forms, a duplication of exon 2 of the constant region was found in the larger message. This was shown to result from a genetic polymorphism in which the gene encoding TCR C_{delta} was duplicated along with some flanking intron sequences. This insertion deletion polymorphism (IDRP) was shown to be inherited as an autosomal codominant trait and animals homozygous for the inserted form had levels of $\gamma\delta$ T cells in their peripheral blood that were equal to rabbits homozygous for the shorter form. Present studies continue to characterize T cell surface markers that may play a role in pathogenic effects of HTLV-I infected cells.

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PROJECT NUMBER

Z01-AI-00170-17

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Functional Analysis of Human Class II Histocompatibility Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Eric O. Long	Section Head	LIG, NIAID
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6	5	1

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many immune responses occur only if a CD4 T lymphocyte recognizes foreign antigen in association with a self class II molecule of the major histocompatibility complex (MHC). Antigen must be processed for presentation by class II molecules and this generally involves endocytosis of antigen into an acidic compartment where newly synthesized class II molecules have been transported. The foreign peptide/MHC class II complexes recognized by CD4 T cells are thus formed intracellularly. The aim of this project is to define the potential sources of antigen for presentation by class II molecules, and to determine how class II molecules are transported to and from antigen-processing compartments. The classical pathway for presentation of exogenous antigen requires newly synthesized class II molecules associated with the invariant chain, and requires HLA-DM, a class II-related molecule encoded in the MHC. However, it was demonstrated here that several processing pathways exist for antigen presentation by class II molecules. First, in contrast to the classical pathway, presentation of an influenza virus antigen was independent of protein synthesis, of the invariant chain, and of HLA-DM. Evidence was obtained that mature class II molecules at the cell surface internalize and recycle to the cell surface. In addition, cytosolic antigen can also be presented to class II-restricted T cells in a manner clearly distinct from the class I pathway, as (i) the invariant chain blocks the presentation of cytosolic peptides by class II molecules and (ii) the transporter for antigen presentation is not required whereas the class II region, including HLA-DM, is. Transport of newly synthesized class II molecules to endosomes is mediated by the invariant chain (Ii), but the intracellular transport route is not known. Using a novel assay based on labeling with galactose and trimming of terminal sialic acid residues with neuraminidase, it has been demonstrated that a large population of class II-Ii complexes reach the cell surface or early endosomes prior to their delivery to late endosomal compartments. The only precedent for retrieval to the cell surface of molecules in late endocytic compartments involves transport through the trans-Golgi network. However, evidence was obtained that class II molecules are transported back to the cell surface by a novel pathway distinct from retrieval through the trans-Golgi network.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00171-17

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infection of Rabbits with Human Immunodeficiency Virus 1

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.J. Kindt	Chief	LIG, NIAID
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	B.F. Hague	Staff Fellow	LIG, NIAID
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4	2	2

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infection in rabbits injected with HIV-1 has been detected by seroconversion, detection of virus in cells and organs using PCR and *in situ* hybridization, and by isolation of virus from PBMC. While the fact that rabbits can be infected with HIV is promising, the slow course of infection, difficulty in isolation of virus and the absence of consistent disease signs in infected rabbits have limited utility of this model for AIDS. Information gained in recent efforts aimed at improving the model suggest that infection in the rabbit may be enhanced in several ways. Adaptation of HIV to grow more efficiently in rabbits has been attempted by long term *in vivo* passage; injection of as little as 1 ml of blood from animals receiving splenocytes from a rabbit infected with HIV-1 and held for 2.3 years has resulted in lymphopenia, CD4 T cell depletion and for 2 of 12 rabbits, death. Initial results from structural analyses of HIV-1 amplified from a spleen cell recipient shows nucleotide sequence identity of 85% to the original input isolate, HIV_{Lai}.

In order to determine whether the slow progression of infection in rabbits is rooted in problems with transcription of viral DNA has prompted preparation of CAT constructs utilizing the 5' or 3' LTRS from HIV-1 to provide the promoter or 3' processing signals. These are being used to compare LTR-mediated gene expression in rabbit and human cells. There are no apparent defects in promoter or processing of genes flanked by HIV-1 LTR's. In subsequent experiments, molecular clones of HIV-1 were used in transfection experiments to assess production of infectious virus in rabbit as compared to human cell lines. Both species produced virus that appeared normal by all criteria, including ability to infect human cells. The rabbit lines produced a somewhat higher ratio of defective particles.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00180-16

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.J. Kindt	Chief	LIG, NIAID
Other:	T.M. Zhao	Staff Fellow	LIG, NIAID
	R.M. Simpson	Expert	LIG, NIAID
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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A series of HTLV-I cell lines were prepared by *in vitro* transformation of rabbit peripheral blood mononuclear cells (PBMC) using HTLV-I cell lines of human or rabbit origin as the virus source. Animals injected with certain infected lines developed high temperatures within four days of injection and died by day 8. The majority of rabbit HTLV-I cell lines, including several derived in the same fashion as the lethal line, cause no overt disease. A detailed study of this phenomenon is underway; results here can shed light on why HTLV-I infection in humans causes serious disease in only a small percentage of infected individuals whereas most infected persons remain asymptomatic. *In vitro* studies show that the lethal cell line causes apoptosis of T lymphocytes whereas other lines activate PBMC in culture. Viral and/or cell components involved in the induction of apoptosis are being studied. Another avenue of investigation involves derivation of HTLV-I molecular clones to use for a study of specific viral genes. One clone was derived from rabbit HTLV-I T-cell line, RH/K30 which contained two copies of integrated provirus; one of these contained an inframe stop codon in the env gene. A genomic fragment containing the other HTLV-I provirus was cloned into lambda phage and sequence determination revealed an intact HTLV-I genome. Several human and rabbit T cell and fibroblast lines transfected with the RH/K30 clone expressed HTLV-I p24 gag protein at levels comparable to the donor RH/K30 line. A transfected rabbit cell line, RL-5/K30, produced type C retrovirus particles with a density of 1.17g/ml; these contained HTLV-I RNA and had reverse transcriptase activity. In addition to HTLV-I expression, RL-5/K30 cells differed from the parent RL-5 line in that RL-5/K30 showed increased expression of CD25 (IL-2ra) levels and expression of surface class II MHC molecules. RL-5/K30 was able to transmit HTLV-I infection as measured by both *in vitro* and *in vivo* assays. Rabbit PBMC and human cord blood mononuclear cells cocultured with irradiated RL-5/K30 cells became infected with HTLV-I. Rabbits injected with RL-5/K30 cells produced an antibody response to HTLV-I proteins, HTLV-I provirus was detected in PBMC beginning 3 months after injection and blood cultures were positive for HTLV-I p24. Availability of such clones will facilitate functional studies of HTLV-I genes and gene products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00389-10

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Geneotype Analyses of HLA and TCR Genes in Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mary Ann Robinson	Section Head	LIG, NIAID
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	H. Deulofeut	Visiting Fellow	LIG, NIAID
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COOPERATING UNITS *(if any)*

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genes encoded within the major histocompatibility complex (MHC) and T cell antigen receptor (TCR) gene complexes have been shown to play important roles in immune responses and in susceptibility to autoimmune diseases. The contribution of individual gene products within these gene complexes and the mechanisms by which they function have not been elucidated. In the present studies, inheritance patterns of HLA and T cell receptor (TCRA and TCRB) genes were analyzed in families. Structural analyses have led to a refined estimate of the extent of the germline TCRBV repertoire and to the identification of polymorphic gene segments and regions. Two frequently occurring insertion/deletion related polymorphisms (IDRP) were found in the TCRB gene complex; one involves a stretch of 21.5kb in the V region and another spans ~20kb near the C region. Three TCRBV genes are encoded in the inserted TCRBV region; BV7S3, BV9S2(P), and a second copy of BV13S2 which is identical to a gene present in all haplotypes. The inserted region shows a pattern of sequence identity with other regions of the gene complex that suggests genetic mechanisms for its origin may include unequal crossing over, gene conversion like events, insertion and deletion of short segments of DNA and mutation. Deleted haplotypes contain 60 genes of which 50 are functional, whereas, inserted haplotypes contained 63 gene of which 52 are functional. Three BV genes were found to have null or unexpressed alleles. The utilized TCR repertoire was analyzed in specific immune responses. The TCR present on T cell lines with defined specificities were characterized and the extent of junctional diversity associated with each TCRBV family was determined. Oligoclonal populations of T cells expressing a few BV families were present on T cells responding to Hepatitis surface antigen. The BV genes used by different responder individuals were similar. Activated cells from patients with autoimmune diseases were analyzed in a similar fashion. Patients with Kawasaki's disease showed oligoclonal expansion of several BV families. Detailed knowledge of the extent and diversity of the germline TCR repertoire will greatly facilitate our ability to understand the role of TCR genes in immune responses in normal and disease states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AI-00525-07

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of Human Killer T Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Nicolai Wagtmann	Visiting Fellow	LIG, NIAID

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W. Biddison, Neuroimmunology Branch, NINDS, NIH.

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4	3	1

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Natural Killer (NK) cells play an important role in the control of viral infection before the establishment of a specific cytolytic T cell response mediated by CD3-positive MHC-restricted T cells. On the other hand, NK cells are involved in the rejection of bone marrow transplants. NK cells must receive a negative signal from target cells in order to spare them from lysis. The precise mechanism of target cell recognition by NK cells is still unknown. The aim of this study was (i) to determine which target cell elements control the recognition and lysis by NK cells, and (ii) to isolate molecular clones for the p58 NK receptor that is involved in transmitting the negative signal upon recognition of HLA class I molecules expressed by the target cells. It was demonstrated that the HLA-B27 allele of class I molecules can provide protection from lysis by a subset of NK clones. Furthermore, analysis of mutated HLA-B27 molecules revealed that different specificities existed even among HLA-B27-specific NK clones and that NK clones must distinguish between different conformations of HLA class I molecules. Purification of the p58 NK receptor was achieved and peptide sequences were derived that could be used to obtain molecular clones for p58 by recombinant DNA technology. The isolation of a complete cDNA encoding one of the members of the p58 receptor family revealed that this receptor represents a new protein belonging to the immunoglobulin superfamily.

LABORATORY OF IMMUNOLOGY
1994 Annual Report
Table of Contents

Z01-AI Project Number		Page
Summary		7 - 1
00030-26	Antigen Recognition and Activation of Immunocompetent Cells - Paul	7 -17
00035-19	Design and Synthesis of Immunomodulators, Immunogens, and Vaccine Constructs - Inman	7 - 18
00036-29	Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System - Mage	7 - 19
00134-32	Control of Immunoglobulin Synthesis in Mice - Asofsky	7 - 20
00224-13	Role of Cytokines in the Pathogenesis and Treatment of Autoimmune Disease - Shevach	7 - 21
00226-13	Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes - Mage	7 - 22
00349-11	Structure and Function of Murine Class II MHC Genes and Gene Products - Germain	7 - 23
00394-11	Molecular Genetic Analysis of Lymphocyte Function - Margulies	7 - 24
00403-11	Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire - Germain	7 - 25
00425-10	Lymphocyte Physiology - Chused	7 - 26
00427-10	Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity - Sitkovsky	7 - 27
00493-08	Interleukin-4 (IL-4) - Paul	7 - 28
00520-07	Analysis of T Lymphocyte Responses to HIV Proteins - Germain	7 - 29
00545-06	Antigen Processing and Presentation - Germain	7 - 30
00565-05	The Family of κB Regulators for Genes in the Immune Response - Lenardo	7 - 31
00566-05	Gene Regulatory Events in Establishing Mature T Cell Tolerance - Lenardo	7 - 32

00622-03	In Vitro Measurement of Binding of Self and Antigenic Peptides to MHC Molecules - Margulies	7 - 33
00623-03	In Vitro Folding and Assembly of MHC Molecules - Margulies	7 - 34
00624-03	New Chemical Tools for Immunology and Structural Biology Research - Inman	7 - 35
00625-03	Protein Phosphorylation in the Effector Functions of T-Lymphocytes - Sitkovsky	7 - 36
00626-03	Inhibitors of Protein Kinases (or Phosphatases) as Immunomodulators - Sitkovsky	7 - 37
00627-03	Regulatory Events in T Cell Development in the Thymus - Lenardo	7 - 38
00628-03	Antigen-Induced Apoptosis (Propriocidal Regulation) of Mature T Lymphocytes - Lenardo	7 - 39
00630-03	Role of $\gamma\delta$ T Cells in Immunoregulation - Shevach	7 - 40

Lay Summary of Laboratory of Immunology Accomplishments- FY93

The Laboratory of Immunology (LI) of the National Institute of Allergy and Infectious Diseases conducts research designed to gain a fundamental understanding of the operation of the immune system. This research places primary emphasis on basic investigation of the development, recognition properties, and regulation of function of immune cells and their products. Acquisition of this knowledge is required to rationally develop approaches to enhance or suppress specific immune responses in humans and to regulate the growth of cells of the immune system. It should have substantial importance to vaccine efforts aimed at controlling many infectious diseases, such as HIV infection and tuberculosis, to attempts to use the immune system to fight cancer, in the design of immune-based therapies for autoimmune diseases such as diabetes, multiple sclerosis, and rheumatoid arthritis, and to attempts to amerliorate allergies.

Among the major accomplishments of LI during FY94 were the following:

The Cytokine IL-5 Plays A Critical Role in Immunoglobulin Isotype Switching

Antibodies perform a variety of important effector functions in immune defense, and also contribute to autoimmune disease and allergy. The variable portions of antibodies control specificity, whereas the effector properties of antibodies are determined by the constant portions of the molecule. The variable portions of antibodies become associated with different effector constant regions by a process of DNA rearrangement known as isotype switching. Past work had suggested that upon B lymphocyte stimulation, the cytokine IL-4 was sufficient to induce a high level of switching to the IgG1 isotype. During the past year, new studies in the Laboratory of Immunology have shown that when B cells are stimulated in a manner more similar to the way they are activated in the body, IL-4 alone is not sufficient to induce this change in isotype, and the cytokine IL-5 is now found to also be a major factor in the switching process. These results are significant not only for the insight they may bring to the detailed molecular mechanism involved in the switching process, but because IL-5 production is frequently high in the airways of asthma patients, they may also indicate how production of the sensitizing IgE antibody class is maintained in these individuals (C. Chu and W.E. Paul, LI/NIAID; R. Mandler and C. Snapper, USUHS; E. Max, CBER, FDA).

Direct Measurement of T Cell Receptor Binding to Peptide-MHC Class I Antigenic Complexes

T lymphocytes are one of the two major sets of cells involved in specific immune responses to foreign substances (antigens). These lymphocytes do not directly recognize the intact molecules of infectious agents or grafted cells, but instead respond to small fragments of proteins called peptides, bound tightly to membrane proteins termed major histocompatibility complex (MHC) molecules. Because T lymphocyte recognition of antigen thus involves two membrane-associated molecules, the T cell receptor and the peptide-MHC molecule complex, it has been difficult to directly measure the interaction of these molecules. Acquiring detailed information on how tightly different peptide-MHC complexes bind to a particular receptor, and the rates of binding and release, is essential to further understanding of how antigen recognition by the T cell receptor is translated into T cell signalling events that control the development of immature T cell precursors and the effector activities of mature T cells. During the past year, LI scientists, in collaboration with investigators at Yale University, developed both a new method for producing soluble versions of T cell receptors and peptide-MHC molecule complexes, and a highly sophisticated and quantitative procedure for measuring how these molecules interact. They were able to analyze this interaction for both the original antigen and variants of the antigen. This allowed them to begin to correlate the different affinities or strengths of interaction with the structure of the peptide-MHC complex and with T cell behavior. These advances in quantitative

analysis of T cell recognition events will contribute greatly to our ability to design optimal vaccine preparations, as well as compounds to modulate unwanted immune responses in patients (D. H. Margulies, LI/NIAID).

Intracellular trafficking and Antigenic Peptide Acquisition by MHC Class II Molecules

Class II MHC molecules have as their function the binding of peptides from the proteins of microorganisms that have been imported into the cell via vesicles that form the endocytic pathway. The precise pathway taken by MHC class II molecules from their site of synthesis to the endocytic pathway remains unknown. This is also true of the place(s) in the endocytic pathway where fragments of antigenic proteins are captured by these molecules for display to T cell receptors after later movement to the cell surface. During the past year, LI scientists developed methods for tracing the movement of class II molecules inside cells and for determining not only their location, but whether or not they had bound antigen. These methods involve breaking up the cell and separating its parts by centrifugation, followed by analysis of radioactively labelled class II proteins. Using this approach, it was possible to demonstrate that class II molecules first move from the site of synthesis in the rough endoplasmic reticulum to the cells surface in the company of a second protein termed invariant chain. They then rapidly enter the endocytic pathway and move through the various early and late parts of the endocytic pathway to a type of immature lysosome, the major protein-degrading organelle of the cell. All along this endocytic route, invariant chain is removed to varying degrees and the class II molecules acquire peptide fragments of antigens. The loaded class II molecules then move back to the cell membrane, possibly via a special sorting station, where they can be recognized by the receptors of CD4+ T cells. These data provide new insight into the precise locations at which antigens are degraded and the resulting fragments bound to class II molecules. The demonstration that there are multiple sites at which this process occurs suggests that different parts of a protein may be released and captured at different locations. This is useful information in the design of optimal methods for delivery of vaccine components to the class II processing system, and may yield insights into how some portions of certain self-proteins are able to stimulate T cells despite the body's many mechanisms for inducing self-tolerance (F. Castellino and R.N. Germain, LI/NIAID).

Control of MHC Class II Assembly, Transport, and Binding Site Function By Invariant Chain

MHC class II molecules must assemble in the endoplasmic reticulum and move to the endocytic pathway free of tightly bound peptides, if they are to properly function in the capture of pieces of internalized antigens in endosomes. During the past year, LI investigators have found that a small part of the larger invariant chain protein contains all the information necessary to support effective assembly of the class II dimer, help it move out of the assembly site, and block its peptide site until it reaches the endocytic pathway. Remarkably, all these functions involve a short region in the protein about the size of an antigenic peptide. Peptide binding to class II molecules is known to help stabilize their structure, and other studies by LI scientists and their collaborators this year have provided evidence that even weak association of peptides with the class II binding region helps preserve the molecule from denaturing at physiological temperature. This has led to the hypothesis that invariant chain works by weakly filling the class II binding site; this helps promote proper protein organization right after biosynthesis and movement inside the cell. This same region is designed to be readily removed from class II in the endosomal pathway, freeing the binding site for capture of antigenic peptides. This model helps relate the behavior of class I MHC molecules, for which peptide binding in the endoplasmic reticulum is critical for assembly and transport, to the properties of class II molecules, and also provides some of the first insights into how different regions of invariant chain help class II perform its functions at different intracellular locations (P. Romagnoli, S. Sadegh-Nasseri, and R. N. Germain, LI/NIAID; L. Stern and D. Wiley, Harvard Univ.).

Programmed Cell Death is Induced in Autoimmune T Lymphocytes By High Antigen Doses

Many cells in the body are eliminated when they have served their function by a process known as programmed cell death or apoptosis. This process involves the active self-destruction of a cell when it receives a specific signal from its environment, or when supporting factors necessary for its survival are not available. This process is well-known to help remove potentially harmful self-reactive T cells from the body while they are differentiating in the thymus. More recently, LI scientists have shown that mature functional T lymphocytes also undergo programmed cell death if their receptors interact with antigen while they are dividing. These observations, made with isolated T cells in tissue culture, suggested that repeated high doses of antigen given to an animal might also lead to the self-destruction of the activated T lymphocytes. This approach could be used as a strategy to selectively destroy T cells responsible for autoimmune diseases. To test this hypothesis, mice were treated to induce a disease called experimental allergic encephalomyelitis (EAE), which is thought to be an animal model of multiple sclerosis. Some animals were given repeated high doses of myelin basic protein, which is the self-antigen to which the autoimmune T cells are reactive. This treatment reduced the incidence and severity of disease. Tests with T cells that could be counted by use of a special antibody showed that the high dose antigen treatment actually eliminated these cells from the body, as predicted from the culture studies. These data provide evidence for new mechanism of immune regulation, and offer the possibility that this method could be adopted for use in treatment of humans with allergic or autoimmune diseases (J. Critchfield and M.J. Lenardo, LI/NIAID).

NF-κC: A Negative Regulator of NF-κB Site Activity

Genetic activity is regulated by DNA binding proteins interacting with specific sequences of nucleotides (bases) in certain regions of each gene. Many of the genes involved in activities of the immune system contain a specific sequence of bases called an NF-κB site. Binding of the NF-κB protein to this site is usually correlated with increases in the activity of the corresponding gene. Variants of this site exist in both the IL-2 gene and the regulatory portion (LTR) of the human immunodeficiency virus (HIV). During the past year, LI scientists discovered that binding of NF-κB to DNA is inhibited by a novel protein complex called NF-κC. This protein is made up of two identical copies of one of the two protein subunits of NF-κB, and it competes with NF-κB for DNA binding, thus inhibiting gene activation by NF-κB interaction with its specific DNA target site. NF-κC was also found to bind to the HIV LTR, suggesting that it might serve as a negative regulator of virus growth in infected cells. Another protein, I-κC, interacts with NF-κC, and keeps it from binding to the NF-κB site, thus relieving the negative effect of NF-κC. I-κC may correspond to the bcl-3 gene; it is produced in response to T lymphocyte activating signals, and presumably works to support IL-2 production, and possibly HIV replication, by inhibiting the repressor NF-κC protein. These data provide new insights into the regulation of a key immune hormone (IL-2) and provide new targets for therapies aimed at controlling HIV replication (M. J. Lenardo, LI/NIAID).

CD4+ NK1.1 Cells: A Critical Source of IL-4 in IgE Responses

The lymphocyte-produced hormone or cytokine IL-4 plays a major role in regulating the quality and vigor of immune responses, especially antibody production. IL-4 is critical for secretion of IgE, the antibodies involved in acute allergic responses and possibly asthmatic attacks. IL-4 also inhibits the development of T cell dependent inflammatory responses. It is thus of substantial importance to understand how IL-4 production is regulated. LI scientists had previously shown that T cell production of IL-4 itself requires the presence of IL-4, raising the question of the initial source of this cytokine. During the past year, LI investigators have

discovered that a novel subset of T cells, termed CD4+ NK1.1 cells, is a major source of IL-4, and secretes the bulk of the IL-4 made in response to primary T cell receptor stimulation *in vivo*. Despite possessing what is usually a marker for cells recognizing MHC class II molecules, the CD4+ NK1.1 cells are absent in mice lacking MHC class I expression as a result of mutation in the β2 microglobulin gene. The function of these cells seems defective in certain mouse strains that are also deficient in IgE production, suggesting that the IL-4 they produce plays a major role in promoting production of this antibody subclass. These results provide a possible answer to the paradox of an IL-4 requirement for T cell IL-4 production, and a new target for the treatment of allergic disease (T. Yoshimoto and W.E. Paul, LI/NIAID; A. Bendelac (LCMI/NIAID).

Manipulation of In Vivo Cytokine Production and Autoimmune Disease

Two opposing classes of lymphoid hormones, or cytokines, are produced by CD4+ T lymphocytes after differentiation. Th1 cells primary produce inflammatory cytokines such as γ-interferon, whereas Th2 cells produce B cell stimulatory cytokines such as IL-4. These key cytokines also regulate each other's production. Thus, understanding how each class of cytokine can be controlled *in vivo* is critical to designing strategies for favoring humoral vs. cell-mediated immune responses. This would permit inhibition of unwanted production of pro-inflammatory cytokines that cause tissue damage in autoimmune diseases, or of the "helper" cytokines that stimulate secretion of the IgE antibodies that produce allergic states. LI scientists have been able to show this past year that administration of bacterial toxin superantigens to animals together with IL-4 results in the production of a large number of IL-4 producing T cells, confirming in a living animal the positive feedback control of IL-4 production seen in cell culture systems. Significantly, these same studies showed that the cells producing IL-4 could be rendered unresponsive if large and repeated injections of superantigen were administered, suggesting that it may be possible to "turn off" IL-4 producing cells in allergic patients using specific antigen under the proper conditions. Conversely, administration of IL-4 together with a self-antigen that normally causes a severe neurologic autoimmune disease in mice led to a reduction in neuron damage and disease severity. This was accompanied by diminished inflammatory cytokine levels in the central nervous system, consistent with the role of IL-4 in opposing such inflammatory cytokine production by T cells, and perhaps guiding newly developing autoreactive T cells into the Th2 (IL-4 producing) rather than Th1 (γ-interferon producing) state. These new findings hold out promise for the use of such" counterbalancing" cytokine therapy in human autoimmune diseases in which inflammatory cytokines play a destructive role (M. Rocken and E. M. Shevach, Cellular Immunology Section, LI, NIAID; M. Racke, Neuroimmunology Branch, NINDS; C. Raine, Div. of Neuropathology, Albert Einstein College of Medicine).

The Ly49 Molecule on Natural Killer Cells Recognizes MHC Class I Molecules

Natural killer (NK) cells are cytotoxic lymphocytes that play a major role in host defense against a number of pathogens and possibly also help control the growth of transformed cells. These NK cells have a poorly characterized recognition system that guides the activity of their cell killing mechanism. Recent studies have suggested an important role for MHC class I molecules in NK recognition of target cells and delivery of effector function. During the past year, LI scientists and their collaborators at Mt. Sinai School of Medicine have been able to use purified soluble candidate NK receptors (the Ly49 molecules) from a subset of natural killer cells together with newly developed soluble MHC proteins and binding assays to directly demonstrate that the class I molecule H-2D^d is a ligand for this NK surface protein. The direct demonstration of this molecular interaction opens the way to careful characterization of whether the peptides bound to the MHC class I molecule affect natural killer cell specificity, and to precise measurement of the binding affinity of the Ly49 and D^d proteins. These studies open the way to a greater

understanding of the natural killer recognition system, with the eventual hope of developing methods of regulating natural killer function and promoting the activity of these key participants in host immune defense (L. Boyd, and D. H. Margulies, Molecular Biology Section, LI, NIAID; W. Yokoyama, F. Karlhofer, Mt. Sinai School of Medicine).

CD28 Costimulation in Expression of the B Cell Activator Molecule CD40L

The human immunodeficiency called hyper-IgM syndrome has been determined recently to be due to mutations in the gene for the T cell-expressed, B cell activating protein CD40L. Because of its critical role in promoting effective immunoglobulin secretion, especially of the IgG and IgA isotypes that have important effector functions in host defense, understanding the regulation of CD40L expression on CD4+ T lymphocytes is fundamental to our knowledge of immune system behavior. During the past year, LI scientists have discovered that the expression of CD40L on T lymphocytes is regulated not only be signals generated by antigen recognition through the T cell receptor, but by costimulatory signals generated by interaction of the T cell surface molecule CD28 and its counter-receptor B7 on the antigen-presenting cell. Because B7 expression is in turn highly regulated, this new finding indicates that a cascade of costimulatory events that begins with activation of the antigen presenting cell determines the capacity of T cells to effectively stimulate B cells to differentiate and secrete various immunoglobulins. This knowledge should be important in vaccine design, and in autoimmune states in which inappropriate CD40L expression might contribute to excessive or pathologic immunoglobulin secretion (L. Ding and E. M. Shevach, Cellular Immunology Section, LI, NIAID).

A Role for Immunoglobulin Variable Region Structure in B Lymphocyte Development

Although antigen-receptor mediated selection events are well-recognized in the development of T lymphocytes, there is little evidence for comparable effects of specific antigen-receptor signaling events in B cell differentiation. LI investigators have now found that during development of B cells in the rabbit appendix, a mutation that affects the expression of a particular immunoglobulin V_H region interferes with B cell differentiation and results in excess B cell death in this organ. This effect seems to be mediated by the absence of a particular structural feature of the V_H portion of the immunoglobulin termed the V region allotype. B cell immunoglobulin receptors lacking this feature seem to be less effective in undergoing clonal expansion and maturation without cell death than B cells with this feature. These data suggest that a type of receptor-related "positive" selection of B lymphocytes occurs during their development. Besides its fundamental significance for understanding lymphocyte differentiation, these data may be helpful in understanding how the repertoire of human B cells is formed and how this repertoire might be manipulated for clinical benefit (R. Pospisil, P. Weinstein, and R. G. Mage, Molecular Immunogenetics Section, LI, NIAID).

PHS-NIH
Summary Statement
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Laboratory of Immunology
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Introduction

The goals of the Laboratory of Immunology (LI) are the elucidation of the fundamental mechanisms of the immune system; the application of the resultant knowledge to the understanding of the pathogenesis of immunologic disorders such as autoimmunity, immunodeficiency, and allergic diseases; and the development of new approaches to immunization that would be useful in vaccine development. LI scientists have made important progress toward these ends through the application of powerful new technologies that have revolutionized modern biomedical science. These include techniques of contemporary molecular biology, cell biology, and protein biochemistry, preparation and study of transgenic and gene-targeted mice, development and use of monoclonal antibodies, cloning and genetic manipulation of normal and transformed lymphocytes, and flow cytometric analysis and sorting. Investigations using these methods have provided answers to many major basic questions in immunology. This new knowledge is contributing to important advances in the control of infectious diseases, as well as to efforts to prevent and treat diseases of the immune system and disorders caused by the action of immune cells and antibodies.

The X-Linked Immunodeficiency of Mice is Caused by a Point Mutation in the Gene Responsible for X-Linked Agammaglobulinemia in Humans

The x-linked immunodeficiency (*xid*) gene of mice results in an inability to make antibody responses to polysaccharide antigens, substantial diminution in serum IgM and IgG3 levels and a 50% reduction in B cell number. In previous studies, the *xid* gene and the gene for a novel tyrosine kinase called *btk* were found to co-map. Because mutations in *btk* are responsible for X-linked agammaglobulinemia (Xla) in man, it seemed quite likely that a defect in mouse *btk* was responsible for the *xid* phenotype. Sequencing the *btk* cDNA derived from CBA/N mice and the closely related normal strain CBA/CaHN revealed a single nucleotide difference resulting in a change at position 28 from arginine to cysteine. This mutation lies in the plekstrin homology domain of *btk*. Surprisingly, the same mutation in the human gene is associated with classical Xla syndrome, implying that there is a significant difference in the function of the *btk* kinase in B cell development or activity in the two species. The mutant *btk* of mice is present in normal amounts and shows normal autophosphorylation activity. This implies that the severe functional defect associated with this mutation may involve either a change in regulation of kinase activity or changes in protein-protein interactions, rather than a simple loss of enzymatic activity. Understanding how the *xid* mutation leads to altered B cell function should help elucidate the physiological role of *btk* in B cells, and aid in understanding the generation of anti-polysaccharide antibody responses that are key in defense against important bacterial pathogens such as pneumococci (J.D. Thomas and W.E. Paul, LI/NIAID; P. Sideras, Univ. of Umea; I. Vorechovsky and C.I.E. Smith, Karolinska Institute; V. Chapman, Roswell Park Memorial Institute).

Diversification of Immunoglobulin Heavy Chain Variable Region Gene Sequences and Allotype-Associated B Cell Selection in the Rabbit Appendix

Although the rabbit immunoglobulin (Ig) H chain genetic region contains multiple variable (V_H) genes, 80 to 95% of the B cells of mature rabbits express immunoglobulin that is derived from the same V_H gene (V_H 1). To generate a broad primary antibody repertoire, this initially

rearranged germline sequence must undergo diversification. Previous studies suggested a central role for gut-associated lymphoid tissues (GALT) in this process, especially the rabbit appendix. Analysis of follicles in the appendix of 6 week old rabbits showed two distinct germinal center zones. Dark zone cells were found to have extensively diversified the CDR2 and CDR3 regions of the Ig heavy chain V region, whereas many of the light zone sequences were closer to germ line. Gene conversion was postulated to contribute to this process along with junctional diversity and somatic hypermutation, and potential gene conversion donors have now been identified among new clones of rabbit immunoglobulin genes. B cells with suitable immunoglobulin receptors may be selected from this pool of variants by recognition of antigen associated with follicular dendritic cells.

A second form of selection also appears to occur in the rabbit appendix during this time period. *Alicia* strain rabbits have a deletion in the J-proximal end of the V_H locus that results in loss the V_H1 gene that is rearranged in most rabbit B cells. Other V_H genes in *Alicia* rabbits mainly lack the V_Ha2 allotype marker. At six weeks of age, *ali/ali* rabbits have higher numbers of apoptotic B cells in the appendix than normals, and show little B cell proliferation in the dark zone compared to normals. The decreased proliferation correlated with the finding that the majority of cycling cells in normal or mutant rabbits had the a2 allotype, and this allotype is underrepresented in young *ali/ali* rabbit B cells. By 11 weeks of age, the mutant rabbits have a cell distribution in the appendix similar to a 6 week normal rabbit. This is paralleled by an increase in a2 positive B cells. These data suggest that the a2 allotype may participate in selection of rabbit B cells during development in the appendix, and that cells bearing receptors with Ig receptors possessing this allotype may receive signals for proliferation and expansion, rather than undergoing programmed cell death. Gene conversion and the expression of V_H1-like upstream V regions in developing B cells of *ali/ali* rabbits seems to account for the gradual appearance of a more normal appendix architecture and lymphoid traffic pattern in older mutant rabbits. These data provide a lead into the possible molecular recognition events involved in B cell amplification and selection in the rabbit appendix (H.-T. Chen, R. Pospisil, P. D. Weinstein, and R. G. Mage, LI/NIAID).

Early Molecular and Cellular Events in Thymocyte Development

The development of mature αβ receptor-bearing T cells involves the intrathymic differentiation of pro T cells via an extended set of intermediate states into so-called single positive CD4 or CD8-bearing lymphocytes. The early precursor cells are characterized by a lack of CD4, CD8, or T cell receptor expression, but other surface proteins such as c-kit have been used to identify and characterize these cells. Surprisingly, a small population of T cell receptor-negative cells bearing the natural killer (NK) marker NK1.1 along with c-kit has been identified that shows MHC-unrestricted cellular cytotoxicity *in vitro* and that can serve as progenitors for both mature CD4 or CD8 T cells and for NK1.1 lymphocytes. These new data imply that the NK and T lymphocyte lineages can develop from a common precursor, and provide an opportunity to determine which factors regulate the lineage choice between the more adaptive T cell and non-adaptive NK cell immune populations. Early T cell precursors lacking T cell receptor expression also show a unexpected expression of a number of DNA binding proteins usually associated with antigen receptor-activated mature T cells, including NF-κB, NFAT, and AP-1. The expression of these nuclear factors declines during further differentiation and then is subject to reactivation in an antigen-dependent manner in the mature progeny of these precursors. The presence of these nuclear factors in immature thymocytes implies active signal transduction by mechanisms other than the conventional antigen receptor pathway. This opens the door to analysis of the early inductive events in T cell development, some of which appear to involve the combination of c-kit ligand, IL-1, and IL-7. These cytokines can induce expression of markers such as CD25 on precursor thymocytes, events that represent one of the first steps in progression to double positive thymocytes that are the substrate for MHC-dependent selection events. Further understanding of

these early inductive stimuli may allow manipulation of T cell development in marrow grafted or T cell compromised individuals, as well as selective promotion of differentiation into the NK vs. T cell lineages (L. Yin, J. Züñiga-Pflücker, Y.-N. Chang., D. Jiang, P. Schwartzberg, and M. J. Lenardo, LI/NIAID; H. Varmus, NCI).

Molecular Genetics of Rabbit Immunoglobulin Gene Rearrangement

The recombination activating locus-1 and -2 (RAG-1 and -2) genes play a central role in the DNA rearrangements that underlie generation of functional B and T cell receptor genes. To further study the role of the RAG genes and their products in the development of rabbit B cell receptor chains and secreted immunoglobulin in this species, RAG-1 and RAG-2 genes were cloned, mapped, and sequenced, and an antiserum to RAG-2 produced. This latter reagent is now being used to characterize the distribution of the corresponding protein in various sites of B lymphocyte development and V gene diversification. Cells expressing RAG genes show active rearrangement of various DNA segments involved in producing functional B cell receptor proteins or secreted immunoglobulin. To examine this process at a molecular level from its earliest stages, DNA circles that result from excision of DNA between rearranging elements in a common transcriptional orientation were isolated and characterized from rabbit bone marrow, a major site of early B cell development. Using PCR based methods, it was possible to determine the frequency of rearrangements of D_H to specific J_H gene segments. The frequencies with which rearrangements occurred to each tested J_H segment were different and did not correlate with sequence overlaps in the D and J coding regions. J_H2 nd J_H4 rearrangements were most frequent, and the latter became overrepresented in the functional Ig of B cells as a result of post-rearrangement selection. These studies are permitting the creation of a detailed picture of the molecular events involved in establishment of the rabbit B cell repertoire and the role of RAG gene products during both early rearrangements and later diversification events (M. Fitts, P. Fuschiotti, and R. G. Mage, LI/NIAID).

Regulation of Cytokine Gene and HIV LTR Enhancer Activity by a Novel Nuclear Complex, NF-κC

κB is a consensus 10 nucleotide sequence found in the regulatory region of a large number of genes of immunological importance, and also in the LTR of HIV. Binding of nuclear factors to κB sites helps to provide cells with temporal and differentiation-state specific control of gene activation. Studies of IL-2 gene activation in T lymphocytes has led to the discovery of a novel factor termed NF-κC that binds to the κB site in the IL-2 promoter/enhancer, and whose binding activity is sensitive to small changes in the nucleotide sequence of κB sites in different genes. This factor is a homodimer of the p50 subunit of NF-κB. It possesses the functional characteristics of a repressor protein, in that its nuclear concentration in decreased by signals that activate the T cell. The decrease is inhibited by cycloheximide and cyclosporin A, and is dependent on another protein termed IκC that acts to block p50 complex binding to the IL-2 κB site. Preliminary data suggest that IκC may be the bcl-3 protein. Intriguingly, NF-κC also binds to the enhancer region of HIV. This result supports a model in which NF-κC inhibits HIV expression in infected cells, and in which T cell activation signals, through the induction of IκC, inhibits NF-κC and allows activation of the HIV LTR. Parallel studies have also shown that the HIV LTR is more active in T_H2 than in T_H1 phenotype T cells, a result that might explain in part the correlation between a switch from T_H1 to T_H2 phenotype during progression to AIDS and an increasing viral burden. These data thus provide new insight into the molecular basis for IL-2 gene regulation in T lymphocytes, and reveal a possible mechanism for control of HIV replication in T cells of distinct activation and differentiation states (D. Jiang, B. Combadiere, and M. J. Lenardo, LI/NIAID).

CD4+ NK1.1 Cells: A Critical Source of IL-4 in IgE Responses

CD4+ T lymphocytes differentiate into effector cells with two major phenotypes. Th1 cells produce the inflammatory cytokines IL-2 and γ-IFN, whereas Th2 cells produce IL-4. The development of each of these differentiated effector states is strongly influenced by the cytokine environment to which the T cell is exposed during antigenic stimulation. Recent studies have shown that IL-12 is an important inducer of the Th1 phenotype; its presence during T cell priming guides differentiation towards γ-IFn production, and inhibits IL-4 secretion. Conversely, this laboratory has previously shown that the presence of IL-4 during T cell activation primes the cells for subsequent IL-4 production upon restimulation, and inhibits the ability of the same cells to produce γ-IFN. Thus, IL-12 and IL-4 are antagonistic cytokines in terms of their influence on differentiation of naive CD4 T cells. A major question has been the source of the IL-4 during an early T cell response. LI scientists have found that a recently described T cell subpopulation termed CD4+ NK1.1 cells is the predominant source of IL-4 immediately after stimulation of animals with anti-CD3 antibody or superantigens *in vivo*. Production of IL-4 by these cells is not itself dependent on IL-4, suggesting that these CD4+ T cells are able to serve as a primary source of this cytokine during immune responses, and thus, to influence the differentiation pathway followed by other T cells upon antigen exposure. The production of IL-4, apparently by these cells, is deficient in SJL mice and mice lacking β2 microglobulin gene expression. These same animals have deficiencies in IgE production, suggesting that the secretion of IL-4 by the CD4+ NK1.1 cells plays a critical role in IL-4 production during immune responses that lead to IgE responses. The identification of this unique cell population as a primary source of IL-4 influencing the development of IgE responses provides an important lead in determining the molecular basis for the defect in IgE responses in SJL mice. It also suggests that further study of this cell population will help provide new insight into the control of this antibody subclass, which plays a major role in allergic disorders in man (T. Yoshimoto and W.E. Paul, LI/NIAID; A. Bendelac (LCMI/NIAID).

The Molecular Basis of IL-4 Receptor Signal Transduction

Many regulatory and effector functions of the immune system depend on the secretion of cytokines, and the generation of intracellular signals in cells whose surface receptors bind these secreted proteins. The action of IL-4 is mediated by binding to a high affinity receptor that is a member of the hematopoietic receptor family. One chain of this receptor binds IL-4 tightly, whereas a second chain common to the IL-2, IL-4, and IL-7 pathways, termed γ_c, plays a major role in the generation of intracellular signals. Stimulation of the IL-4 receptor leads to phosphorylation of a homolog of the major substrate of the insulin receptor. This protein, termed 4PS, is tyrosine phosphorylated when IL-4 binds to its receptor. This biochemical effect is lost if the cytoplasmic tail of the IL-4 receptor is deleted. The critical component of the IL-4 receptor tail involves a tyrosine residue that is part of a sequence of amino acids homologous to the sequence around a critical tyrosine in the cytoplasmic tail of the insulin and IGF-1 receptors, now termed the I4R motif. A fusion protein containing this motif binds both the 4PS substrate and a kinase that phosphorylates the 4PS protein. Thus, the I4R region appears to serve as a docking site for both the substrate and kinase. This new findings should enable isolation of the kinase and permit the further elucidation of the cascade of biochemical events responsible for transducing IL-4 binding into gene regulatory events in the target cell. Because of the central role of IL-4 in immune regulation, this knowledge will be of eventual use in designing new drugs that regulate IL-4's effects. It also should help unravel the mechanisms by which receptors using a common subunit (γ_c) transduce different signals in the same cell (A. Keegan and W.E. Paul, LI/NIAID; J. Pierce and L-M. Wong, NCI; S. Russell and W. Leonard, NHLBI).

The Role of IL-5 in Immunoglobulin Class Switching in Antigen-Receptor Activated B Lymphocytes

Immunoglobulin class switching is a process that occurs in individual activated B lymphocytes that allows the development of cells secreting diverse antibody isotypes from a precursor expressing only immunoglobulin of the IgM class. An especially well-studied example of this event is the switch in mouse lymphocytes from IgM to IgG1 production. Previous *in vitro* studies using the polyclonal B cell mitogen LPS as a stimulant provided evidence for a model in which LPS endowed the B cell with the capacity to undergo isotype switching, whereas the cytokine IL-4 was involved in targeting the switch mechanism to the γ1 constant region locus. Evidence for DNA deletion in the chromosomal region between the switch (S) regions 5' to the Cμ and Cγ1 loci was obtained from a newly developed digestion/circularization PCR (DC/PCR) technique. This method involves digestion of B cell DNA with restriction enzymes that cut adjacent to the S regions, followed by ligation of the digested DNA at low concentration to form circles. A PCR product is formed using primers complementary to sequences 5' of Sμ and Sγ1 only if a chimeric S region was generated in the B cells used as a source of DNA. This method has now been applied to a study of class switching in B cells activated in a more physiologic manner through anti-immunoglobulin cross-linking of their antigen receptors, rather than by use of LPS. Surprisingly, a combination of anti-surface IgM antibodies and IL-4 led to little IgG1 production, even though the IL-4 exposure could be shown to have prepared the cell for switching, based on production of RNA transcripts of the unrearranged Cγ1 locus. This lack of switching was confirmed at the DNA level using DC-PCR, which showed no evidence of recombination after exposure to anti-IgM and IL-4. Addition of IL-5, however, led to substantial IgG1 secretion, as well as Sμ/Sγ1 recombination assayed using DC-PCR. These results indicate an important role for IL-5 in activating a component of the class switching mechanism, and further studies are in progress to identify the molecular basis of this effect (C. Chu and W.E. Paul, LI/NIAID; E. Max, CBER, FDA; R. Mandler and C. Snapper, USUHS).

The Role of B7 Costimulation in T Cell Expression of the B Cell Activating Molecule CD40L

Activation of B lymphocytes for the secretion of immunoglobulins of diverse isotype depends on the T cell expression of the CD40L (gp39) molecule, a member of the TNF family of cell-associated cytokines. Defects in this protein in man result in the immunodeficiency disease termed X-linked hyper-IgM syndrome. CD40L is not expressed by resting T cells, but is upregulated during activation. LI scientists have found that the induction of CD40L expression during antigen-dependent stimulation of CD4+ T cells is dependent on costimulatory signals delivered by the B7/CD28 pathway. This contribution of the CD28 pathway to CD40L expression was detected using an RT-PCR method for evaluation of CD40L mRNA levels, an approach that circumvents the problem of CD40L modulation or blocking that occurs in the presence of the CD40 receptor present on the antigen presenting cell. Although B7/CD28 signalling clearly was involved in CD40L upregulation, the soluble fusion protein CTLA-4Ig that interrupts this pathway was only able to inhibit CD40L upregulation by 50-70%, suggesting that additional costimulatory pathways can facilitate the expression of CD40L. Because CD40L plays a crucial role in antibody responses, especially those involving isotype switched immunoglobulin secretion, the demonstration that the B7/CD28 pathway is involved in CD40L induction is an important step towards understanding the steps involved in translating peptide-MHC molecule display of antigen into a functional effector immune response (L. Ding and E. M. Shevach, LI/NIAID).

Regulation of Basophil and Mast Cell IL-4 Secretion by FcγRII Binding of IgG1

Mast cells and basophils secrete significant amounts of IL-4 and other immunoregulatory cytokines upon cross-linking/aggregation of their Fc receptors. Human basophils grown in

culture with IL-3 secrete IL-4 in response to cross-linking the FcγRII, but not FcγRI or FcγRIII receptors. The nature of the ligand engaging the FcγRII appears to determine the basophil response. Thus, immobilized IgG4 is a potent stimulant of IL-4 production whereas immobilized IgG1, although able to bind to FcγRII, fails to initiate such IL-4 secretion. In fact, soluble IgG1 is as potent an inhibitor of IL-4 secretion in response to immobilized IgG4 as soluble IgG4 itself. Similarly, soluble immune complexes of IgG4 induce IL-4 production, whereas soluble complexes of IgG1 alone do not. Although IgG1 cannot act as an agonist in this system on its own, it is able to modify the response to IgG4. Thus, at limiting amounts of immobilized IgG4, moderate to high amounts of IgG1 can strikingly enhance the IL-4 response. These data suggest that IgG1 can function as either an antagonist or as a partial agonist for IgG4-dependent, FcγRII-mediated IL-4 production. Because IgG4 is the least abundant serum IgG, this effect of IgG1 at limiting IgG4 concentrations may significantly amplify the activity of low amounts of IgG4 antibody in the induction of mast cell and basophil IL-4 production. This effect may be of significance in allergic states and/or during responses to helminths (H. Eidelson and S. Z. Ben-Sasson, Hebrew Univ. and W.E. Paul, LI/NIAID).

Regulation of In Vivo Cytokine Production

Cytokines are major mediators on both immune protection and immune system dependent diseases such as allergies, asthma, and autoimmunity. Although a substantial amount is known about the regulation of cytokine production by T cells *in vitro*, less is known about the *in vivo* control of the production of these mediators. LI scientists have developed a model system for examining the induction of T cells producing the key lymphokine IL-4 *in vivo*. Using the superantigen SEA to activate cells, and manipulating the dose of superantigen and coadministration of IL-4, these scientists have been able to show that moderate doses of SEA plus IL-4 leads to the generation of large numbers of primed cells capable of producing IL-4 in response to superantigen restimulation. However, repetitive administration of SEA abolished the ability of naive or primed SEA-specific cells to produce IL-4. This unresponsiveness could be reversed by infection with the nematode *N. brasiliensis*, but not by administration of superantigen and IL-4. These data provide some of the first evidence for the existence of a "tolerant" or "unresponsive" state for IL-4 producing cells *in vivo*; this finding may be of significance in the design of treatments for allergic diseases resulting from IL-4 promotion of IgE production. At the same time, the ability of IL-4 to promote cells to produce IL-4 *in vivo* suggested the possibility that administration of this cytokine could induce differentiating autoreactive T cells seeing self-antigen *in vivo* to produce IL-4 at the sites of inflammatory autoimmune disease, and downregulate such inflammatory responses. This model has been tested by coadministering IL-4 with primed, pathogenic autoantigen-reactive T cells in a mouse model of multiple sclerosis termed experimental allergic encephalomyelitis. This approach led to the amelioration of disease, with reduced demyelination, and diminished inflammatory cytokine production in the CNS. These therapeutic effects correlate with the development of Th2-like autoantigen-specific T cells in the host animal. Together with the data on tolerance induction in IL-4 producing cells, these results suggest that *in vivo* manipulation of T cell cytokine production is possible, and provide starting points for the further development of strategies that modulate cytokine expression to reduce allergic or autoimmune diseases (M. Rocken, A. Bonomo, and E. M. Shevach, Cellular Immunology Section, LI, NIAID; M. Racke, Neuroimmunology Branch, NINDS; C. Raine, Div. of Neuropathology, Albert Einstein College of Medicine).

In Vitro and In Vivo Evidence for Antigen-Induced Apoptosis of Mature T Lymphocytes and the Application of This Phenomenon to Regulation of T Cell-Mediated Autoimmune Disease

Programmed cell death or apoptosis is a major mechanism for physiologic control of the size of differentiated cell populations. Apoptosis induced by antigen receptor-dependent

stimulation of cycling cells has been shown by LI investigators to represent a potent means of limiting clonal expansion of mature T lymphocytes. In the past year, direct evidence has been obtained that so-called "high dose suppression", an *in vitro* phenomenon in which increasing concentrations of antigen lead to progressively lower proliferative responses from cloned CD4+ T lymphocytes, results from such receptor-signal induced apoptosis or "propriocidal" cell death. This propriocidal death is a consequence of apoptosis induced by high levels of antigen receptor signaling during the S phase of the cell cycle, an event that is common among T cells in a culture stimulated with high concentrations of cognate ligand. The recognition that high dose suppression involves antigen-induced cell death provides a possible explanation for the recent reports of extensive clonal T cell depletion in animals subject to strong and prolonged antigen challenge *in vivo*. This model also predicts that intentional exposure of an animal to extended high level antigenic challenge could provide a means of selective depletion of disease-inducing autoreactive T cells. This hypothesis was tested using the experimental allergic encephalomyelitis (EAE) model. Whether induced by adoptive transfer of primed CD4+ T cells or by immunization with myelin basic protein in Freund's adjuvant, the severity and incidence of EAE in mice was reduced by repetitive injection of the animals with high doses of MBP. This effect could be correlated with depletion of MBP-specific T cells using transfer of monoclonal T cells reactive with MBP from a T cell receptor transgenic mouse. Similar preliminary data have been obtained in an experimental uveitis model and a model of egg allergy. Taken together, these data extend the concept of propriocidal regulation of T cell function to relevant *in vivo* models of T cell disregulation. They suggest that further studies are warranted in the use of this approach in the selective destruction of disease-causing T cells in patients with autoimmune diseases, as well as in investigations of the reasons for immune failure in defense against various micro-organisms that give rise to high antigenic burdens (S. Boehme, L. Zheng, H. McFarland, J. Critchfield, M. Freedman, S. Batra, J. Milner, T. Yoo, and M. J. Lenardo, LI/NIAID; M. Racke, C. Pelfrey, H. McFarland, Neuroimmunology Unit, NINDS; R. Caspi, LI, NEI; J. Governman, Univ. Washington).

Invariant Chain Control of MHC Class II Assembly, Transport, and Peptide Binding

MHC class II α and β chains typically assemble with the non-MHC encoded type II glycoprotein invariant chain shortly after cotranslational import into the endoplasmic reticulum (ER). Previous studies in transfected cells and mice made deficient for invariant chain expression by gene targeting have shown that invariant chain plays a major role in the efficient assembly of certain class II heterodimers and their movement from the ER through the Golgi complex, while at the same time inhibiting antigenic peptide binding. LI scientists have now shown that the role of invariant chain in class II heterodimer assembly involves releasing the molecules from high molecular weight aggregates which form immediately upon chain import into the ER. These aggregates have a very short half-life (several minutes) in normal cells producing both class II chains and invariant chain, whereas the class II α and β chains remain largely in such aggregates for hours in cells lacking invariant chain expression. Even those heterodimers that do form in the absence of invariant chain transit out of the ER very inefficiently. The regions of invariant chain contributing to these early post-synthetic events, and to the inhibition of peptide binding, were analyzed using a series of deletion mutants. Remarkably, elimination of the entire C-terminal half of invariant chain had no detectable deleterious effect on its participation in these events. Removal of the exon 3 encoded region (residues 83-107), however, led to a failure of the remaining short piece of invariant chain to associate with class II or to mediate any of these functions. This region of invariant chain corresponds precisely to the so-called CLIP peptide that can be eluted from purified class II molecules, especially those produced in cells lacking DM expression. Taken together, these data suggest a model in which the CLIP region acts as a surrogate ligand for the class II binding site. This helps stabilize and properly fold the heterodimer, while at the same time inhibiting binding of either antigenic peptides, or more importantly, peptide-sized stretches of amino acids in the folding intermediates of the large number of proteins in the ER. Thus, invariant

chain provides for class II many of the functions that peptide binding in the ER provides for class I molecules, yet does so without permanently inactivating the binding site that needs to be available in the endosomal pathway for peptide antigen capture. These results provide significant new insight into the class II antigen presentation pathway, as well as an intriguing model system for studying protein folding and chaperon interactions. They also may provide insight into the possible role of differences in invariant chain expression and interaction with different MHC alleles on the presentation of endogenous self-proteins by class II (P. Romagnoli and R. N. Germain, Lymphocyte Biology Section, LI, NIAID; M. Marks, C. Bonnerot, and J. Bonifacino, CBMB, NICHD).

Endosomal Localization and Peptide Binding Compartments of MHC Class II Molecules

In contrast to MHC class I molecules that acquire their peptide ligands in the ER, MHC class II molecules present antigenic peptides derived primarily from proteins entering the endocytic pathway. Although this feature of class II antigen presentation has been clear for some time, the precise route taken by class II molecules to reach the endocytic pathway, the site(s) that they accesses, and the location(s) at which they acquire peptide antigen remain poorly characterized. To address these issues, a combination of metabolic pulse-labelling, immunoprecipitation, and subcellular fractionation were applied to normal B cell blasts in an effort to trace the route of class II from the trans-Golgi network (TGN) to the cell surface, and to delineate the compartment(s) in which peptide binding occurs. Using this approach, LI scientists have found that class II-invariant chain complexes first move from the TGN to early endosomes, most likely via the plasma membrane. They also access subsets of vesicles with the properties of late endosomes (transferrin-negative, M6PR+, lamp-1 low) and early lysosomes (lamp-1 high, M6PR negative, hydrolase rich). Invariant chain is removed in proportion to the proteolytic properties of these compartments and the extent of their acidification, and also as a function of time. In the presence of the protein antigen hen egg lysozyme, SDS-resistant class II dimers known to contain processed antigen can be found in all these endosomal fractions. However, the extent of compact dimer formation and the kinetics of this process differ in the three fractions, consistent with a model in which invariant chain removal is a precursor to peptide loading, and in which lack of early invariant chain removal results in the accumulation of the associated class II in the lysosome-like compartment, where subsequent invariant chain dissociation either results in peptide binding or the denaturation/ degradation of the empty class II molecules. Loaded molecules transit to the plasma membrane, possibly via an intermediary compartment of low density that might be related to that recently described by several laboratories. These data provide clear evidence that class II accesses multiple endocytic subcompartments in normal physiological antigen presenting cells, and that the molecules in these various subcompartments can all acquire actively processed antigen. They also begin to define the routing of class II in the cell, from TGN, through the cell surface to early endosomes, and then to more proteolytic and acidic compartments. These data suggest that different antigenic determinants may be processed and captured by class II in distinct endosomal compartments, and that differences in the rate of intercompartmental transit and proteolytic capacity may give rise to distinct antigen presentation capacities in various subpopulations of class II expressing cells, such as B cells, macrophages, and dendritic cells. They also provide the starting point for physical isolation of the class II-containing compartments and a detailed characterization of the other proteins in these compartments that may provide auxiliary functions during class II antigen presentation (F. Castellino and R. N. Germain, Lymphocyte Biology Section, LI, NIAID).

Two Distinct Affinity and Structural States for Peptide-MHC Class II Complexes

The interaction of peptides with MHC class II molecules is unusual in that it is characterized by a combination of a very slow apparent on rate and a very slow off rate. The molecular explanation for this behavior has not been elucidated. Based on the prior observation of

a fast on-fast off form of peptide class II interaction in a single peptide-MHC class II combination, and the recent demonstration of a structural change accompanying class II binding of certain peptides, LI scientists proposed that the slow formation of long-lived peptide MHC class II complexes reflected the rare transition of unstable class II-peptide complexes to a stable structural state. During the past year LI scientists have used a novel soluble form of empty human lass II molecules produced in insect cells to demonstrate that virtually all class II molecules can bind a single peptide in either a fast on-fast off state, or a slow on-slow off state. Thus, the differences between the two forms of binding do not reflect separate populations of class II molecules with different structural characteristics, but the alternative behavior of a given class II molecule upon meeting peptide antigen. The newly reported crystal structure of class II suggests that a large number of both side chain and backbone bonds must be made for tight peptide binding by class II molecules. The kinetic data suggest that many peptides first interact with class II in a manner permitting only a few of these bonds to be formed. Some peptides can reach the optimal binding state by direct rearrangement from this weak binding state; many others apparently dissociate before this can occur and undergo repetitive rounds of interaction with class II, with a subset attaining the stable state in each binding cycle. This accounts for the slow accumulation of long-lived class II-peptide complexes. Surprisingly, the ability of class II molecules to bind peptide was found to be lost as a function of time at 37°C unless the class II molecules were kept in an occupied state by low affinity (fast on-fast off) interactions at high peptide concentrations. This denaturation of empty class II is reminiscent of the instability of class II dimers produced in cells lacking invariant chain, and might indicate that invariant chain occupancy can help stabilize newly formed class II heterodimers against denaturation in living cells until they reach the antigen-rich endosomal environment in which peptide capture is designed to take place (S. Sadegh-Nasseri and R. N. Germain, Lymphocyte Biology Section, LI, NIAID; L. Stern and D. Wiley, Harvard University).

Interactions of αβ T Cell Receptors with Purified MHC Class I Molecules and Peptides

MHC class I molecules containing bound peptide antigen are the ligands for αβ receptors on CD8+ T lymphocytes. Despite years of study, few quantitative measurements of the interactions in this ligand-receptor system exist. Using recombinant DNA methods, LI scientists have been able to produce large amounts of soluble MHC class I proteins, and to selectively load these molecules with defined peptides. They have also worked with scientists from other institutions to produce soluble forms of the αβ receptor from the 2C CD8 T cell clone. Using these reagents and a new method for real-time quantitative analysis of protein-protein interactions (surface plasmon resonance), it has been possible to examine the on and off rates for interaction of a single T cell receptor with its known peptide-MHC ligand, as well as with other class I proteins, and variant peptides. These studies have revealed that at least for this receptor ligand system, TCR interaction with its cognate agonist peptide-MHC class I complex is a rapid on-rapid off ($t_{1/2} \sim 26$ seconds) event. These data suggest that T cell signal transduction depends on the participation of other molecules that stabilize the T cell-antigen presenting cell, and perhaps more importantly, the T cell receptor- MHC ligand interactions, so that long-term second messenger generation can proceed. These studies have also shown the exquisite specificity of the T cell receptor for its ligand, as even small changes in the peptide bound to the correct MHC class I molecule results in a loss of measurable affinity. The development of methods for quantitative measurement of receptor-ligand interactions for T cells opens up a new field of study with relevance to understanding how immune recognition proceeds and results in the differentiation and effector function of these cell (M. Jelonek, M.P. Corr, L. Boyd, C. Hoes and D. H. Margulies, Molecular Biology Section, LI, NIAID; A. Bothwell, Yale University).

The Ly49 Molecule on Natural Killer Cells Recognizes MHC Class I Molecules

Natural killer (NK) cells can be divided into subsets based on the expression of surface

proteins that have properties suggesting they serve as recognition receptors for these cytotoxic effector cells. The Ly49 molecule on mouse NK cells correlates with the MHC class I molecules expressed by the host. LI scientists have now established using mice transgenic for the MHC class I protein H-2D^d, that the presence of the Ly49+ subset of NK cells depends on the absence of this particular MHC class I molecule. Using purified soluble labelled D^d, preliminary data suggest direct binding of this class I molecule to Ly49, a result confirmed by measurements of the interaction of soluble forms of these two proteins using surface plasmon resonance. Intriguingly, the peptide content of the D^d molecules seems to play a role in Ly49 interaction. These studies provide direct evidence of a molecular interaction between a putative NK receptor and an MHC molecule ligand, and establish conditions necessary for examination of the quantitative and qualitative aspects of this recognition event that is key to the operation of the early defense functions of the NK lymphocyte subset (L. Boyd and D. H. Margulies, Molecular Biology Section, LI, NIAID; W. Yokoyama, F. Karlhofer, Mt. Sinai School of Medicine).

Structural and Kinetic Analysis of MHC Class I-Peptide Interactions and MHC Class I Folding

Although substantial information is available on the molecular details of peptide-MHC class I interactions from crystallographic studies, there are few studies available that combine structural analysis with accurate quantitative measurements of binding kinetics and affinity. Using engineered soluble MHC proteins, the technique of surface plasmon resonance, and computer modeling, LI scientists have begun to decipher the details molecular details of MHC class I-peptide interactions. Analysis of a large number of distinct MHC class I - peptide combinations for binding on and off rates have shown that the binding event is slow relative to classical diffusion-limited interactions, suggesting that effective peptide binding requires structural "adjustment" of the MHC molecule. Further evidence for structural changes in MHC class I molecules upon peptide binding were obtained from studies of the serological behavior of either soluble class I heavy chain-β2 microglobulin complexes, or single chain class I proteins that have β2 microglobulin covalently linked to the heavy chain. In the former case, loading peptide into L^d molecules promoted formation of the 30-5-7 epitope, which was not seen with empty molecules. Remarkably, removal of peptide with high pH did not lead to the loss of the 30-5-7 determinant, indicating a form of "molecular memory" that indicated changes in the conformation of the class I protein following peptide binding. Alternative states of folding were also seen when solubilizing bacterially produced single chain class I molecules; in the presence of peptide, the refolded proteins acquired α1.α2 domain specific antibody reactivity, while in the absence of peptide, soluble molecules were produced that lacked these determinants. The former molecules are biologically active and can stimulate T cells, and mice transgenic for such single chain class I molecules have been produced and are being studied. The latter, alternatively folded molecules do not act as effective agonists for T cells primed to natural class I proteins. These quantitative studies on peptide-MHC class I interaction and the detailed analysis of class I structural behavior in the presence and absence of peptide are providing new insights into the process by which class I molecules form effective ligands for CD8 T cells, and have implications for understanding the normal immune response to class I-presented peptide antigens as well as for the development of soluble MHC proteins for use in immunotherapy or vaccination (L. Boyd, S. Khilko, S. Sakuma, xxx and D. H. Margulies, Molecular Biology Section, LI, NIAID; L. Lee and M. Mage, LB, NCI).

Detection of Active CD8+, Class I-Specific Cytolytic T Lymphocytes in β2 Microglobulin-Deficient Mice

The small soluble protein β2 microglobulin (β2m) is an integral part of class I major histocompatibility complex (MHC) class I molecules. Homozygous disruption of the β2m gene in mice leads to the almost complete abrogation of surface class I expression, and a concomitant

decrease in CD8+ T cell differentiation in the thymus. These β2m-deficient animals have been used in numerous studies to investigate the contribution of CD8+ T cells to immune defense against a variety of pathogens. LI investigators have made the surprising observation that such mice seemed to reject MHC class II-negative allogeneic tumor grafts whose elimination was considered to be the result of cytotoxicity mediated by CD8 + lymphocytes. Analysis of cells from mice having rejected such tumors showed the presence of highly cytolytic effector cells bearing CD8 and the αβ T cell receptor, despite earlier studies suggesting that β2m deficient animals lacked such cells due to a failure of thymic positive selection. Transfer of these cells into nude mice conferred significant protection against tumor grafts. In contrast to normal animals, the generation of this response was delayed in time and more dependent on host CD4+ T cells than in wild-type mice. These data suggest that either a low level of class I expression in the thymus in the absence of β2m is sufficient to allow very weak but significant CD8+ T cell selection, or that class I independent extrathymic development of such cells occurs. In either case, these results raise an important caution against overinterpretation of data from studies on β2m-deficient mice with respect to the role of CD8+ cytotoxic T cells in pathogen defense or tumor or tissue graft rejection, and indicate that direct tests for such effector cells during an ongoing response are necessary to rule out the development of a cryptic CD8 response in such animals (S. Apasov and M. V. Sitkovsky, LI/NIAID).

Participation of Integrins in Thymocyte Development and Antigen-Independent T Cell Activation

The integrin superfamily consists of a large number of heterodimeric integral cell surface molecules capable of interaction with both extracellular matrix components and other cell surface molecules. LI scientists have recently found that the vitronectin receptor on preactivated mature T cells can stimulate cytokine production by interaction with its ligand in the absence of T cell receptor antigen recognition. This signalling depends on the expression of the ζ chain that is usually found associated with the T cell receptor-CD3 complex and is known to participate in tyrosine kinase-mediated signal transduction events in T cells. Such antigen-independent stimulation of primed T cells might contribute to chronic inflammatory processes. Binding to the extracellular matrix protein fibronectin, and expression of α3β1 and α6β1 integrin receptors was also found to be characteristic of subsets of developing human thymocytes. Changes in fibronectin binding accompanied maturation of thymocytes, while engagement of the latter two integrins costimulated antigen-receptor dependent thymocyte proliferation. These data suggest a complex role for a series of integrins on developing T cells, and together with the data on vitronectin activation of mature T cells, emphasizes the critical contributions of these surface proteins to T cell differentiation in response to the thymic or peripheral stromal environment (C. Brando and E. M. Shevach, Cellular Immunology Section, LI, NIAID; K. Sturmhofel, A. Chang, J. Coligan, LMS, NIAID).

MHC Class-Independent Lineage Commitment of CD4+CD8+ Thymocytes

The two major subsets of αβ receptor-bearing T cells (CD4+ and CD8+) develop from precursors expressing both CD4 and CD8. The mature CD4+ and CD8+ T cells possess receptors that recognize peptides presented by either class II or class I MHC molecules, respectively. This pattern of response correlates with the ability of CD4 to bind to relatively monomorphic regions of class II molecules and of CD8 to bind to monomorphic sites on class I molecules. The differentiate events and signals that determine the MHC class coordination seen on mature T cells between clonotypic T cell receptor specificity and CD4 or CD8 coreceptor specificity remain in dispute. One model proposes that the selective, simultaneous engagement of the TCR and CD4 with class II or the TCR and CD8 with class I on a double positive (CD4+CD8+) precursor cells "instructs" the cell, by virtue of particular intracellular signal transduction events, to turn off the coreceptor not involved in the recognition event, and to mature along the CD4 or CD8 pathway, respectively. In

this model, lineage commitment is concomitant with positive selection for maturation. A second model proposes that the CD4+CD8+ precursor loses expression of one or the other coreceptor and commits to a particular differentiation pathway independently of the MHC class specificity of its receptor; a subsequent receptor:coreceptor engagement event is postulated to be critical for full development into a mature T cell, and this event can only occur if the coreceptor whose expression is retained has an MHC class specificity that matches the peptide-MHC specificity of the clonotypic receptor. This "non-instructional" model thus also gives rise to mature T cells with coordinate TCR and coreceptor class specificity, but it is based on separate lineage commitment and positive selection events. LI scientists studying so-called transitional thymocytes passing from the double positive stage to the single positive stage found that the generation of cells with partially downregulated CD4 expression appeared in mice lacking the MHC class I molecule expression predicted to be involved in "instructing" CD4 coreceptor downregulation. Further examination of class II deficient animals showed that this was true of the reciprocal population as well. These transitional cells had intermediate levels of TCR expression, and a distinct population of $CD4^{intermediate}CD8^{hi}TCR^{hi}$ cells was identified that only appeared in mice with class I molecule expression capable of effective CD8+ T cell selection. Mice lacking both class I and class II expression did not develop transitional cells. These data led to the hypothesis that double positive cells, upon receptor engagement with either a class I or class II ligand, enter into the CD4 or CD8 lineages without regard for the class of MHC molecule (or coreceptor) involved in this signaling event. The resulting transitional cells are then further tested for receptor signaling, which occurs efficiently only when the highly expressed coreceptor matches the MHC specificity of the TCR. These results allow the isolation of highly characterized thymocyte subpopulations for genetic studies on the molecular basis of lineage commitment and positive selection, as well as implying the absence of unique coreceptor dependent signal transduction events in movement out of the double positive thymocyte state (J. van Meerwijk and R. N. Germain, Lymphocyte Biology Section, LI, NIAID; B. J. Fowlkes, LCMI, NIAID).

Ectokinase and Ectophosphatase Activities of Cytotoxic T Lymphocytes

Phosphorylation and dephosphorylation of proteins are known to be among the major mechanisms of regulating cell function. Such events are usually considered to involve the intracellular domains of proteins, and for membrane proteins, to modulate interaction with intracellular signal transduction substrates in particular. Because of prior studies in the LI on a role for extracellular ATP in cell-mediated cytotoxicity, studies on the possible occurrence of ectodomain phosphorylation and dephosphorylation events were undertaken. These experiments have revealed substantial phosphorylation of numerous cell surface proteins at sites external to the cell membrane, as detected by label transfer from exogenously added [γ-P^{32}]ATP and susceptibility of the resultant labelled protein to dephosphorylation by extracellular phosphatase addition. The ectokinase activity appears to be predominantly associated with a casein-kinase II-like enzyme, although other kinases with distinct substrate specificity also appear to be present on the cell surface. These phosphorylation events can be induced in a trans-manner during the interaction of a CTL with its target cell, and many inhibitors of casein kinase II-type enzymes inhibit cell-mediated cytotoxicity. Such cell-cell interaction also allows the activity of ectophosphatase(s) of the PP1 class. Taken together, these data suggest the possible existence of an extracellular kinase/phosphatase system that plays a role in cell-cell interactions, especially during CD8 T cell effector activity (M. V. Sitkovsky, LI/NIAID).

High Molecular Weight Thrombospondin Peptide Conjugates That Inhibit Tumor and Endothelial Cell Growth

Many important biological activities involve small regions of larger proteins that can be mimicked by synthetic peptides equivalents. Unfortunately, *in vivo* use of such free peptides as

drugs is constrained by their rapid clearance/destruction, and by the limited biological activity of unconstrained, monomeric peptides. One such peptide corresponds to an 18-residue segment in the type 1 repeat of human thrombospondin-1, which shows an ability to block the growth of human melanoma, breast carcinoma, and normal epithelial cell lines *in vitro*, and to inhibit AIDS Kaposi's sarcoma cell and breast carcinoma cell growth *in vivo*. The use of this peptide is severely compromised by its tendency to aggregate when synthesized, and to adhere to storage vessels, making it unsuitable for further development as a possible therapeutic agent. Using the expertise developed in the Bioorganic Chemistry Section over many years in the production of polymeric immunomodulatory agents, soluble high molecular weight conjugates of this peptide and Ficoll (polysucrose) were prepared and shown to be 10 fold more potent that free peptide *in vitro*. *In vivo*, this material was highly effective in controlling either Kaposi's sarcoma cell or breast carcinoma cell growth in nude mice. Newer versions of this polymer conjugate involving D-amino acid retro-inverso analogs of the thrombospondin-1 peptide are biologically active and should have an enhanced *in vivo* half-life, due to reduced susceptibility to proteolytic attack. Additional modifications to enhance coupling efficiency and reduce the TGF-β stimulatory potential of this material have been prepared and are undergoing testing. These results suggest that such peptide-polysaccharide conjugates may prove to be highly active agents with the potential to regulate tumor cell growth or neovascularization *in vivo* (J.K. Inman, LI/NIAID; H.C. Krutzsch and D.D. Roberts, LP, NCI).

Honors, Awards and Scientific Recognition

Laboratory of Immunology scientists play important roles in the U.S. and international scientific communities. They serve on editorial boards of many scholarly publications, on the advisory and scientific review boards of major organizations, act as organizers and major participants in international and national scientific meetings, and provide expert advice to numerous biotechnology and pharmaceutical organizations.

LI scientists have major editorial responsibilities for scientific journals and publications. Dr. Ethan Shevach serves on the editorial boards of the *Journal of Immunological Methods*, *Cellular Immunology*, and the *Journal of Biomedical Science*. He is section editor for immunology of *Life Sciences* and an editor of *Current Protocols in Immunology*.

Dr. William Paul is the editor of the *Annual Review of Immunology* and of the advanced textbook *Fundamental Immunology*, the third edition of which was published this year. He is an advisory editor of the *Journal of Experimental Medicine*, an associate editor of *Cell* and an editorial board member of *Immunity* and *Immunological Reviews* . He is also a transmitting editor of *International Immunology* .

Dr. Ronald Germain is an advisory editor of the *Journal of Experimental Medicine* and a member of the editorial board of *Immunity*. Dr. Rose Mage is a member of the editorial board of *Immunogenetics*. Dr. David Margulies is an editor of *Current Protocols in Immunology* and a Section Editor for the *Journal of Immunology*. Dr. Michail Sitkovsky is an associate editor of the *Journal of Immunology*. Dr. Lenardo is a member of the editorial board of *Molecular and Cellular Biology*. Dr. John Inman is a member of the editorial board of *Analytical Biochemistry* and is an advisory editor of *Molecular Immunology*.

Dr. Paul is a member of the Medical Advisory Board of the Howard Hughes Medical Institute and of the Advisory Committee of the Department of Molecular Biology, Princeton University. He also is a jury member for the Albert Lasker Medical Research Awards, a member of the Awards Assembly for the General Motors Cancer Research Award, and chair of the Selection Committee, Jesse Stevenson Kovalenko Award of the National Academy of Sciences. He was awarded an honorary fellowship from the American Academy of Allergy and Immunology, elected a fellow of the American Association for the Advancement of Science, and presented with the Award of Honor from Brooklyn College.

Dr. Shevach is a member of the Scientific Advisory Board of the American Leprosy Foundation, a member of the fellowship review subcommittee of the American Heart Association, and a member of the Steering Committee on Transdisease Vaccinology, Global Program for Vaccines, WHO.

Dr. Germain received the DHHS Distinguished Service Award. He served as membership chairman of the American Association of Immunologists and as a member of the Scientific Advisory Boards, Ruggiero Ceppellini Advanced School of Immunology, Naples, Italy, Roche Milano Ricerche, Milan, Italy, and MedImmune, Inc., Gaithersburg, MD. He is also a member of the Human STD Challenge Study Advisory group, NIAID, NIH.

Dr. Lenardo received the USPHS NIH Director's Award. Dr. Rose Mage serves as adjunct professor for the George Washington Univ. Genetics Program and was a site visit member, CBER FDA Laboratory of Molecular Immunology. Dr. David Margulies is on the study section for Immunotherapry of the Multiple Sclerosis Foundation. Dr. Sitkovsky received the USPHS Service Award.

Dr. Paul delivered the Blumenthal Lecture in Allergy and Immunology at the Univ. of Minnesota, and spoke in the Distinguished Scientists Lecture Series at the Univ. of Maryland School of Medicine. He also was an invited speaker at the Fourth Annual Symposium of the Johns Hopkins Asthma and Allergy Center, the Combined Meeting of the 8th International Lymphokine Workshop and 4th International Workshop on Cytokines in Osaka, Japan, the Symposium in Honor of Michael Sela's 70th Birthday, Weizmann Institute of Science, Rehovot, Israel, the Ein Gedi Symposium "Molecular Mechanisms and Physiological Activities of Cytokines", Ein Gedi, Israel, and the 5th International Conference on Lymphocyte Activation, Newport Beach, CA.

Dr. Germain was an invited lecturer at the Univ. of Iowa, Dept. of Internal Medicine. He presented plenary lectures at the American Academy of Dermatology Annual Meeting, a symposium on the Immunoglobulin Supergene Family: Structure, Function, Idiotypes, and Disease, Maui, Hawaii, a Symposium on the Immunoglobulin Gene Superfamily in Autoimmune Disease, Univ. of Pennsylvania, the 9th H2/HLA Workshop, Garda, Italy, the UCLA Keystone Symposium of Lymphocyte Activation, Keystone, CO, the Symposium on Antigen Processing and Presentation at the FASEB meeting, Anaheim, CA, and the US-Japan Meeting in Hamilton, MO. He also was an invited lecturer in the NIH InterInstitute Structural Biology Lecture Series, and spoke at the NIAID-sponsored Educational Symposium at FASEB.

Dr. Margulies was an invited lecturer at Pennsylvania State University Medical School, the US-Japan Immunology Meeting in Hamilton, Montana, the XVIIth International Union for Biochemistry and Molecular Biology Meeting in New Dehli, India, the 9th H2-HLA Workshop, Garda, Italy, and the 1994 Biosensor meeting in Baltimore, MD.

Dr. Lenardo presented lectures at the Sloan-Kettering Cancer Center, the University of Chicago, and Northwestern University. He was an invited speaker at the NIAID DAIDS workshop "HIV-mediated defects in immune regulation", a symposium on "κB-binding proteins - their role in development and growth control", Cold Spring Harbor, NY, the Clinical Immunology Course on "Immunological aspects of AIDS", the IBC Conference on Transcription, Washington, DC, the Ein Gedi Symposium "Cytokines and the Immune Response", Ein Gedi, Israel, the Cambridge Health Institute Conference "Apoptosis", San Diego, CA, and 5th International Conference on "Mechanisms of Lymphocyte Activation and Immune Regulation", Newport Beach, CA.

Dr. Shevach spoke at the 12th Meeting of the International Society of Hematology, Vienna, Austria, at the annual meeting of the Swiss Society of Immunology, Basel, Switzerland, and the International Workshop on Paroxysmal Nocturnal Hemoglobinuria, Pathophysiology and Biology of GPI-Anchor Structures, Hanover, Germany.

Dr. Mage lectured in FAES Immunology classes and at the Univ. of Mississippi.

Administrative, Organizational and Other Changes

Early this year, Dr. William Paul, Chief of the Laboratory of Immunology, was appointed Director, Office of AIDS Research and Associate NIH Director for AIDS Research. To assist in the administrative operation of the Laboratory of Immunology during Dr. Paul's tenure in these posts, Dr. Ronald Germain, Chief of the Lymphocyte Biology Section, was appointed Deputy Laboratory Chief.

The Laboratory of Immunology also completed a world-wide search to fill a tenure-track position whose incumbent will conduct both investigator-initiated research and supervise a gen-targeting service facility for NIAID. Dr. Hua Gu, who became a leader in the area of targeted gene disruption and replacement during his postdoctoral time in the laboratory of Dr. Klaus Rajewsky in Cologne, Germany, was offered and accepted this post. He is currently in the process of beginning research in his new laboratory at Twinbrook II, and establishing a gene targeting service facility at the same location. We anticipate that Dr. Gu will be a major contributor to the application of this exciting methodology to investigation of lymphoid development and immune system function. We also expect that through collaboration with Dr. Gu and with the help of the service facility, investigators throughout NIAID will be able to bring the application of this powerful technology to bear on many diverse questions of interest.

The Laboratory of Immunology is a major training center for young immunologists. During the past year, several scientists completed post-doctoral training or sabbatical periods in the Laboratory. Among these are Adriana Bonomo, Clara Brando, Pierpaolo Correale, Jeffrey Critchfield, Matthew Fitts, Matthew Freedman, Steven Kozlowski, Andrew Lees, Jacqueline Little, Nobuhiro Ohori, Shigeru Ohno, Peter Rabinovich, Sheherezade Sadegh-Nasseri, Linda Sakamura, Shozo Sakuma, Jeffrey Thomas, and Maurice Zauderer. Each of these individuals made important contributions to the Laboratory of Immunology research program. It is anticipated that they will have very productive research careers and that many will attain leadership positions in modern immunology or related field.

During the past year, several scientists joined the Laboratory of Immunology as postdoctoral fellows or as students. They include Hangjiong Chen, Behazine Combadiere, Galen Fisher, Ricai Han, Hugh McFarland, Paul McGovern, Elisabeth Payer, Daniel Plaksin, Katarina Polakova, Fiona Topfer, and Guangming Zhong. It is expected that they will continue the tradition of excellence established by a long series of trainees in the Laboratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00030-26 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William E. Paul	Chief	LI, NIAID
Others:	Jeffrey Thomas	Guest Researcher	LI, NIAID
	Charles Chu	Staff Fellow	LI, NIAID
	John Ryan	Staff Fellow	LI, NIAID
	Hang-Jiong Chen	Visiting Fellow	LI, NIAID
	S. Z. Ben-Sasson	Visiting Scientist	LI, NIAID

COOPERATING UNITS *(if any)*

CBER, FDA (E. Max); Uniformed Services University of the Health Sciences (R. Mandler, C. Snapper); Roswell Park Memorial Institute (V. Chapman); Karolinska Institute (C. I. E. Smith and I. Vorechovsky); Univ. of Umea (P.Sideras); Hebrew Univ./Hadassah Medical Center (S. Z. Ben-Sasson)

LAB/BRANCH

Laboratory of Immunology

SECTION

General Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.5	1.5

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A specific and quantitative assay for measuring the frequency of chromosomes in which Ig class switch events have occurred has been developed. This quantitative and specific assay, digestion-circularization PCR (DC-PCR), allows one to measure the frequency of chromosomes on which deletional switching has occurred. A critical role for the cytokine interleukin-5 (IL-5) in the initiation of the class switch event has been demonstrated in B cells stimulated with anti-immunoglobulin antibodies and interleukin-4 (IL-4). Although IL-4 targets certain switch regions for recombination, virtually no recombination events occur unless IL-5 is also present. Efforts to analyze the molecules induced by IL-5 that control recombination are now in progress.

The X-linked immunodeficiency of CBA/N mice has been shown to be caused by a point mutation in the gene for the tyrosine kinase *btk* that converts amino acid 28, within the plekstrin homology domain of the kinase, from arginine to cysteine.

Basophils and mast cells secrete IL-4 and other cytokines as a result of cross-linkage of Fc receptors and elevation of intracellular calcium. Human basophils secrete IL-4 in response to cross-linkage of FcγRII. However, this receptor transduces distinct signals depending upon the ligand. Thus, immobilized IgG4 induces IL-4 production while immobilized IgG1 does not although it does bind to the receptor and, under very specific conditions, enhances IL-4 production in response to immobilized IgG4. This indicates that IgG1 may act as an antagonist or a partial agonist depending upon conditions, which may have important physiologic significance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00035-19 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Design and Synthesis of Immunomodulators, Immunogens, and Vaccine Constructs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John K. Inman	Section Chief	LI, NIAID
Others:	Patricia F. Highet	Technician	LI, NIAID

COOPERATING UNITS *(if any)*

Food & Drug Admin (B. Golding, H. Golding); State University, Utrecht, The Netherlands (H. Snippe); Lab. Pathology, NCI, NIH (H. C. Krutzsch, D. D. Roberts); Dept Microbiol. Immunology, Virginia Commonwealth University (K. L. McCoy)

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	0.8	0.8

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed toward the design and synthesis of immunogens, vaccine constructs, immunomodulators and biological response modifiers for collaborative studies of both basic and applied nature. Much of this work has been focused upon (1) studying immune responses to *Brucella abortus* conjugated with gp 120 or V3-loop peptides derived from human immunodeficiency virus (HIV-1) strains, with the goal of developing a therapeutic vaccine, (2) developing novel polymer-peptide conjugates that inhibit tumor and endothelial cell growth with potential for the treatment of disease conditions that depend on neovascularization, such as melanomas, breast carcinoma, Kaposi's sarcoma and diabetic retinopathies. Emphasis has been placed on studying the effects, *in vitro* and *in vivo*, of polyvalent presentation of biologically active molecules (peptides, lipopolysaccharides, ligands, haptens, etc.), covalently linked to soluble high molecular weight polymer or protein carriers. New reagents and methods have been developed in this section for synthesizing novel conjugates used for these studies.

Conjugates of HIV-1 derived peptides covalently linked to heat-inactivated *B. abortus* have been found by collaborative investigators at CBER, FDA to elicit virus neutralizing (syncytia inhibiting) antibodies, predominantly of IgG2a isotype, as well as peptide-specific cytotoxic T cells, even in mice severely depleted of CD4+ helper T cells. These constructs are being actively investigated as potential therapeutic vaccines for treating AIDS patients.

Collaborative studies are being carried out with investigators in the Laboratory of Pathology, NCI, on biological responses to synthetic peptides linked to soluble polymer carriers. The sequences are taken from the extracellular matrix protein, thrombospondin-1. A number of variants of a peptide from the type 1 repeat unit have been synthesized and covalently coupled to highly branched polysucrose (Ficoll™). *In vitro* and *in vivo* experiments have shown that many of these conjugates are potent inhibitors of endothelial and tumor cell growth; and thus, they may be useful in blocking metastatic growth of solid tumors. A US patent has recently been filed based on these findings.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00036-29 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Visiting Scientist	LI, NIAID
	P. D. Weinstein	Special Volunteer	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID
	R. Pospisil	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(If any)*

ARD, USAMRID, Ft. Detrick, Frederick, MD (A.O. Anderson); Dept Biol Sci, FL State U., Tallahassee, FL (K. Roux); Dept Path/Lab Med, Univ FL Coll Med., Gainesville, FL (W.T. McCormack); CBER, DCB, FDA (N. Harindranath); Dept Tumor Cell Biol., St. Jude Children's Res Hops, Memphis, TN (L.M. Hendershot); Dept Microbiol. Med Coll Ohio, Toledo, OH (D.W. Metzger); Spring Valley Labs, Inc (R. Shaw)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We used techniques of immunogenetics and molecular biology to study the genes for rabbit immunoglobulins, and other genes including the RAG-1 and RAG-2 which are necessary for gene rearrangements to occur during lymphocyte development. We investigated the development of anatomical sites such as appendix follicles and germinal centers in gut associated lymphoid tissues and the regulated expression and sequence diversification of Ig genes during lymphoid cell development. Lectins allowed us to dissect rabbit appendix follicles and germinal centers into subcompartments based on local expression of glycosylated cell surface receptors. We proposed that in early development, the functional role of the rabbit appendix may be similar to that of the chicken bursa or sheep ileal Peyer's patch. The rabbit appendix, however, does not involute but may change from a site of primary variable region diversification to a site of secondary lymphoid responses. IgM associated B cell receptor molecules were found on rabbit B cells as heteromeric structures with nonreduced molecular weights of approximately 75 kDa and 135 kDa composed of 37 and 42 kDa subunits. Immunological studies with monoclonal antibodies suggested that these proteins are the rabbit homologues of murine Ig-β (B29) and Ig-α (mb-1). Whereas B cells with rearranged VH1 predominate in normal rabbits, in homozygous *Alicia* mutant rabbits (*ali/ali*) the VH1 gene is deleted and B cells with upstream VH genes rearrange. We found differences between appendix cells from normal and *ali/ali* rabbits based on immunohistochemistry patterns and cell trafficking analyses. The trafficking patterns of appendix cells found in 6 week normals were similar to those found in 11 week old *ali/ali*. A higher proportion of B cells expressing the a2 allotype may receive strong signals to survive rather than undergo apoptosis. The a2 allotype probably plays functions role(s) in selection and effective expansion of B cells in the appendix.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00134-32 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	Richard Asofsky	Section Chief	LI, NIAID
Others:	J. Little	Staff Fellow	LI, NIAID
	N. Ohori	Visiting Fellow	LI, NIAID
	A. Brooks	Biological Technician	LI, NIAID

COOPERATING UNITS *(If any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TH 2.2 is a cloned hybrid B lymphocyte line which is induced by bacterial lipopolysaccharide (LPS) to secretion of IgM and the cytokines GM-CSF, IL-3, and IL-6. LPS also markedly inhibits growth of TH 2.2 both in bulk and in clonal culture. Pretreatment of TH 2.2 with 1 or 5 μg/ml of LPS prevents inhibition of growth by subsequent treatment. Responses recover, but require long term passage to do so. Almost all fresh clones of TH 2.2 are sensitive to LPS. Repeated analysis of >60 clones showed that occasional resistance appeared in one passage generation only in 10-15%, and disappeared with subsequent testing. Resistance could be induced in stably sensitive clones of TH 2.2 by treatment with LPS. About 1/3rd of 90 clones from cells pretreated with 1 or 5 μg LPS were fully resistant when first tested. The majority of these clones became slowly sensitive with subsequent passage; 4 have remained resistant, but at lower levels than initially, and four partially resistant clones have remained partially resistant. The effects of pretreatment on cytokine secretion are more complicated: TH 2.2 in short term passage is rendered unresponsive (marked reduction in secretion) by pretreatment; cells in long term passage are rendered hyper-responsive in this respect. In either case, the cells return to normal with long enough further passage.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00224-13 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Receptors, Co-Receptors, and Counter-Receptors for T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	L. Ding	Visiting Fellow	LI, NIAID
	V. Rostaphshov	Visiting Associate	LI, NIAID
	C. Brando	Visiting Associate	LI, NIAID
	C. Mojcik	Staff Fellow	LI, NIAID
	R. Ortmann	Staff Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LMS, NIAID, NIH (J. Coligan, K. Sturmhofel, A. Chang)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The interaction of the T cell receptor (TCR) with its specific ligand, peptide-MHC, is only one component of the events required for the activation of antigen-specific T cells. The primary goals of our studies are to characterize cell surface antigens (co-receptors) and their soluble or cell associated ligands (counter-receptors) which play critical roles in cell-cell interaction and in the interaction of T cells with their environment. We have concentrated our efforts in a number of distinct areas: 1) We have shown that the interaction of the B7 family of antigens on antigen presenting cells (APC) with their co-receptors on T cells is critical for the activation of T cells and that this interaction can be mediated by expression of the B7 antigen on an APC separate from the APC presenting the nominal antigen ("trans costimulation"). The B7 family also was shown to play a critical role in the induction of CD40L, the counter-receptor for the B cell antigen CD40. The interaction of the CD40L with CD40 plays a critical role in T-B collaboration. 2) We have defined a new pathway of non-specific activation of T cells which is mediated by the Vitronectin Receptor (VNR), a member of the integrin superfamily. Ligation of the VNR on certain activated T cells could induce cytokine production by T cells that required expression of the ζ-chain, one of the components of the CD3 complex, but did not require occupancy of the TCR by its cognate antigen. The antigen-independent, TCR-dependent, VNR-mediated pathway may play an important role in chronic inflammation. The rapid induction of tyrosine phosphorylation of a 115 kD protein was shown to be one of the major effects of engagement of the VNR. 3) Continued studies of the role of the integrins in thymocyte development demonstrated that human CD4+CD8+ thymocytes could be divided into two major populations based on their differential capacity to adhere to fibronectin (FN). The FN-adherent population is less mature, expresses higher levels of CD4/CD8, and low levels of the TCR and CD69. In contrast, the non-adherent cells appear to have undergone positive selection and express lower levels of CD4 and/or CD8, high levels of the TCR, and high levels of CD69. It appears that the differentiation of the adherent population to the non-adherent is one of the critical steps of human thymocyte differentiation. Lastly, we have demonstrated that all human thymocyte subpopulations express two receptors (α3β1 and α6β1) for the extracellular matrix proteins, laminin and merosin. Furthermore, adhesion to these proteins can be induced by activation of the thymocytes and both laminin and merosin can costimulate thymocyte proliferation in the presence of suboptimal TCR stimulation.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00226-13 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. G. Mage	Section Head	LI, NIAID
Others:	M. Fitts	IRTA Fellow	LI, NIAID
	H.-T. Chen	Visiting Scientist	LI, NIAID
	P. D. Weinstein	Special Volunteer	LI, NIAID
	P. Fuschiotti	Visiting Associate	LI, NIAID
	R. Pospisil	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(If any)*

ARD, USAMRID, Ft. Detrick, Frederick, MD (A.O. Anderson); Dept Biol Sci, FL State U., Tallahassee, FL (K. Roux); Dept Path/Lab Med, Univ FL Coll Med., Gainesville, FL (W.T. McCormack); CBER, DCB, FDA (N. Harindranath); Dept Tumor Cell Biol, St. Jude Children s Res Hosp, Memphis, TN (L.M. Hendershot); Dept Microbiol, Med Coll Ohio, Toledo, OH (D.W. Metzger); Spring Valley Labs, Inc (R. Shaw)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Immunogenetics Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues x (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We study genes of the rabbit immune system by techniques of molecular biology and immunology. We obtained new data that support the proposal made many years ago that the rabbit appendix could be a bursal equivalent. The DNA sequences of rearranged heavy chain variable region genes from B cells in the light and dark zones of appendix germinal centers from 6-week-old rabbits were highly diversified in CDR2, probably through both gene conversion and somatic hypermutation; some LZ sequences were closer to germline. We derived simple evolutionary trees that showed the relationships of cells with different sequences to each other and to single progenitors. Potential donors that could have participated in gene conversion-like alterations of rearranged genes were identified. We prepared a cosmid library containing 35-45 kb fragments of rabbit genomic DNA in order to further characterize VH gene expression and diversification in the rabbit. We found and are characterizing germline DH and JH genes, as well as upstream VH genes that may be donors for gene conversions. PCR allowed us to specifically identify excision circles resulting from DH to JH and Dβ to Jβ DNA rearrangements. Extrachromosomal circular DNA purified from rabbit bone marrow cells was assayed by PCR to determine the relative in vivo rearrangement frequencies of Ig DH to JH genes. DH genes rearranged to individual JH genes with different frequencies. This bias did not correlate with potential sequence overlaps in the DH or JH coding sequences. The JH2 and JH4 genes were the preferred targets of recombination in primary rearrangements. Analyses of genomic VDJH indicated that B cells expressing VDJH4 heavy chains survived and dominated in the bone marrow due to post-rearrangement selection. Circular products reflecting DβJβ rearrangements were detected in the appendix of adult animals. We described the rabbit RAG locus, genomic and expressed RAG-1 and RAG-2 genes and produced anti-RAG-2 antibodies.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00349-11 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	S. Sadegh-Nasseri	Special Volunteer	LI, NIAID
	R. Han	Visiting Associate	LI, NIAID
	S. Fleury	Visiting Fellow	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	A. Rinker, Jr.	Research Technician	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

Harvard University (L. Stern; D. Wiley); University of Texas Galveston (R. Koenig)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	2.8	.8

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Class II major histocompatibility complex (MHC) gene products play critical roles in a variety of T and B lymphocyte responses. Biochemical and functional analyses have been used to investigate the relationship between class II structure and peptide antigen presentation. The kinetics of class II peptide binding have been examined and two distinct phases of binding identified; a rapid initial binding with a rapid off rate, and a slow accumulation of long-lived binary complexes. Low affinity binding was unexpectedly found to preserve class II molecules from denaturation at physiological temperature, a result with implications for the function of invariant chain (see Z01 AI 00545-05 LI). The direct demonstration of different affinities of interaction of class II with the same peptide suggests that optimal alignment of peptide side chain and class II binding pockets is an infrequent event that provides a kinetic limit to the rate of high affinity peptide capture by class II molecules. These new insights into the biochemical behavior of class II help provide a deeper understanding of how antigen capture by class II molecules occurs under physiological conditions.

In addition to binding peptide and being recognized by clonally distributed T cell receptors, class II molecules participate in T cell selection in the thymus and mature T cell activation in the periphery by interacting with the CD4 molecule that is also the receptor for HIV-1. We have used site-directed mutagenesis to define the site(s) of interaction of class II molecules with CD4. In addition to our previous identification of a major binding site in the β2 region of the class II molecules, we have now located a second important site in the α2 domain. These two sites on a single class II heterodimer cannot both bind to an individual CD4 molecule simultaneously. The two sites do lie close together in the recently published crystal structure of the class II "dimer of dimers". Taken together, these results provide the first evidence for organization of higher order oligomeric signal transduction complexes involving the T cell receptor and CD4 coreceptor. These latter findings, in concert with our studies of T cell signal transduction upon exposure to distinct peptide-MHC ligand complexes (see Z01 AI 00403-11 LI), are defining the molecular mechanisms involved in MHC-dependent antigen recognition by, and activation of T lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00394-11 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Charles Hoes	Biologist	LI, NIAID
	Rosemarie Hunziker	Sr. Staff Fellow	LI, NIAID
	Marie Jelonek	Medical Staff Fellow	LI, NIAID
	Daniel Plaksin	Visiting Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

MB, NCI (J. Berzofsky); LB, NCI (M. Mage, L. Lee); Mt. Sinai School of Medicine (F. Karlhofer and W. Yokoyama); Univ California-SF (B. Daniels and W. Seaman); Univ California-SD (M. Corr); Yale University (A. Bothwell); Boston University (R. Brower and C. DeLisi)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of these studies has been on the functional role of the MHC class I molecule and the development of model systems for examining this function both *in vitro* and *in vivo*. Specific accomplishments during the past year include: 1) quantitative measurement of the interaction of MHC class I molecules-peptide complexes with T cell receptors; 2) the evaluation of the role of the MHC class I molecule H-2D^d in the signalling of a subset of NK cells bearing the cell surface marker Ly-49; 3) the quantitative evaluation and mathematical modeling of the contribution of MHC and peptide to T cell stimulation; and 4) the production of single chain MHC class I molecules for functional and binding studies.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00403-11 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	J. van Meerwijk	Visiting Fellow	LI, NIAID
	J. Madrenas	Visiting Associate	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	A. Rinker, Jr.	Research Technician	LI, NIAID
	L-Huang	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

LCMI, NIAID (B. J. Fowlkes); CMBM, NICHD (L. Samelson; Univ. of Naples (L. Racioppi); LI, NIAID (R. Seder, W. Paul, L. Ding, E. Shevach)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	2.3	1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues x (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antigen-specific T lymphocytes activation occurs through the clonally distributed T cell receptor (TCR), which is involved in thymocyte selection and peripheral T cell effector responses. How TCR-dependent lineage commitment from a common precursor occurs for the two major subsets of T cells, the CD4+ and CD8+ cells, remains unknown. Furthermore, the quantitative and qualitative relationships between receptor occupancy and signaling for differentiation are also poorly understood. This project uses cellular, biochemical, and molecular approaches to study differentiation of thymocytes and activation of T cells upon ligand engagement.

Our previous studies suggested that thymocyte development could involve lineage choice that is independent of the MHC class involved in T cell recognition, followed by complete maturation (positive selection) of only those cells whose remaining expression of CD4 or CD8 matched the MHC class-specificity of the T cell receptor. Studies of additional gene targeted and transgenic mice support this conclusion, yet show that MHC recognition per se is critical to this step in development. These experiments also indicate that the transitional phenotype cells whose presence suggests lineage commitment are not the products of negative selection. These cellular studies will be critical for future molecular studies aimed at the development of a genetic understanding of the basis for T cell differentiation.

We previously reported that some TCR ligands evoke only a subset of T cell effector responses, and that such variant ligands can selectively antagonize cytokine production induced by known receptor agonists. We have now identified additional peptide-MHC class II complexes with such partial agonist/partial antagonist properties. Preliminary studies on the biochemical events that accompany TCR recognition of such variant ligands suggest that they induce the generation of only a subset of the second messengers typically produced upon agonist recognition. These findings, in concert with our work on the molecular organization of T cell signal transduction complexes (Z01 AI 00349-11 LI), will help explain how protein-protein interactions result in antigen receptor-dependent second messenger generation, and how specific intracellular signals contribute to downstream gene activation events in T cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00425-10 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lymphocyte Physiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. M. Chused	Senior Investigator	LI, NIAID
Others:	Shigeru Ohno	Visiting Fellow	LI, NIAID
	R. L. Lampman	Biologist	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Transmembrane Signalling Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues x (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular free ionized calcium, and intracellular pH, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized. These studies have revealed extensive !feed-forward and !feed-back regulatory relationships between ion channel opening, membrane potential, activity of the calcium pump, and rate of phosphatidyl inositol turnover. These mechanisms differ in the T, B and monocyte/granulocyte lineages. The process of signal transduction in thymocyte differentiation is being examined.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00427-10 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	S. Apasov	Visiting Associate	LI, NIAID
	P. Smith	Research Associate	LI, NIAID
	Pearl Chen	Research Associate	LI, NIAID
	Pierpaolo Correale	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	4	

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues x (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The antigen-specific cytotoxicity mediated by cytolytic T-lymphocytes (CTL) is an important part of cellular mechanisms of anti-viral and of anti-tumor immune responses. Functions of CTL depend on their activation after the recognition of target cells which express peptide antigen in their major histocompatibility complex (MHC) molecules and on the susceptibility of target cells to the "lethal hit"- induced programmed cell death (apoptosis). Studies of CTL functions and development are now in a most interesting phase due to the latest breakthroughs in the development of different gene "knock-out" mice. These studies offer the best hope to define the cells that most responsible for destruction of tumors or of virus-infected cells.

Using mice with genetically disrupted β2-microglobulin loci (β_2m-/- mice) and with a deficiency in MHC class I expression, we unexpectedly found in contrast to published reports that intraperitoneal immunization with tumor cells results in the accumulation of highly lytic and MHC class I-specific CD8+ CTL. These studies helped to demonstrate that CD8+ CTL develop even under conditions of very low level MHC class I expression, but impairment of negative and positive selection results in the generation of CTL with an unusually self-reactive recognition repertoire.

Additional insights into T-cell development are being gained from studies of p53 gene (tumor suppressor gene) knock-out mice. We took advantage of the high incidence of lymphomas in these mice and established immortalized thymocyte cell lines (more than 100 different clones representing thymocytes at different stages of differentiation) for detailed studies of T-cell development.

One of the spin-offs of the studies of the mechanisms of CTL-induced cell death is the promising strategy we have developed to achieve immunospecific tumor cell destruction using low concentrations of chemotherapeutic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00493-08 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interleukin-4 (IL-4)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	William E. Paul	Chief	LI, NIAID
Others:	Achsah D. Keegan	Staff Fellow	LI, NIAID
	Keats Nelms	Special Volunteer	LI, NIAID
	Tomohiro Yoshimoto	Visiting Fellow	LI, NIAID
	John Ryan	Staff Fellow	LI, NIAID
	S. Z. Ben-Sasson	Visiting Scientist	LI, NIAID
	R. Germain	Deputy Laboratory Chief	LI, NIAID

COOPERATING UNITS *(if any)*

LCI/NIAID (R. Seder); LCMI/NIAID (A. Bendelac); National Heart, Lung and Blood Institute (S. Russell and W. Leonard); National Cancer Institute (J. Pierce and L-M. Wong): Joslin Diabetes Center (M. White)

LAB/BRANCH

Laboratory of Immunology

SECTION

General Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	3.5	1.5

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Naive CD4+ T cells produce interleukin-2 (IL-2) upon stimulation by antigen and antigen-presenting cells but can be induced to differentiate into cells that principally produce interleukin-4 (IL-4) or interferon gamma (IFNγ). The determination of which lymphokine is dominantly produced has a major impact on the protective value of the immune response. Utilizing naive CD4+ T cells from T cell receptor transgenic mice, it was shown that IL-4 itself is the principal factor determining whether T cells develop into IL-4 producers; in addition, IL-4 strikingly suppresses priming for IFNγ production. In the absence of IL-4, priming leads to cells that produce moderate amounts of IFNγ. Addition of IL-12 to the priming culture upregulates priming for IFNγ production and allows such priming to occur even in the presence of IL-4. The physiologic source of IL-4 that determines this pattern of differentiation is a matter of great importance. A specialized population of splenic CD4+ T cells expressing the NK1.1 marker has been identified and shown to promptly produce IL-4 upon *in vivo* activation with anti-CD3 antibody or superantigens. Two strains of mice (SJL and β2-microglobulin knockout mice) have both a deficiency in IL-4 production by these cells and impaired differentiation to the production of IL-4 and to the secretion of IgE implying that these cells have an important role in the production of IL-4 at the outset of immune responses.

IL-4 action is mediated by binding to a high affinity receptor. This receptor consists of two chains. One binds IL-4 with substantial affinity and also determines the specificity of the signalling pathway. The other (the γ chain of the IL-2 receptor or γc) is essential for transducing biochemical signals. The binding of IL-4 to the IL-4 receptor complex results in the phosphorylation of a 170 kDa substrate, designated 4PS, an analog of insulin receptor substrate-1 (IRS-1). Phosphorylation of 4PS/IRS-1 is lost in truncation mutants of the receptor that delete the most membrane proximal tyrosine. The sequence around that tyrosine is homologous to the sequence around a comparable tyrosine in the insulin and IGF-1 receptors; this motif has been termed the I4R motif. A fusion protein containing the I4R motif binds the substrate and the kinase that can catalyze the phosphorylation of IRS-1. The I4R motif appears to act as a docking site for 4PS/IRS-1.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00520-07 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of T Lymphocyte Responses to HIV Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	A. Fox	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

MB, NCI (J. Berzofsky, T. Takeshita)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
- (b) Human tissues
- (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00545-06 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen Processing and Presentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Germain	Section Head	LI, NIAID
Others:	P. Romagnoli	Visiting Associate	LI, NIAID
	G. Zhong	Special Volunteer	LI, NIAID
	F. Castellino	Visiting Associate	LI, NIAID
	C. Reis e Sousa	Visiting Fellow	LI, NIAID
	L-Y. Huang	Research Technician	LI, NIAID
	A. Fox	Research Technician	LI, NIAID
	A. Rinker, Jr.	Research Technician	LI, NIAID

COOPERATING UNITS *(if any)*

CBMB, NICHD (M. Marks, J. Bonifacino); Harvard University (E. Bikoff)

LAB/BRANCH

Laboratory of Immunology

SECTION

Lymphocyte Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.9	3.9	1.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

αβ T cells respond to peptide-MHC molecule complexes. How protein antigens are transformed into peptides suitable for such binding and the intracellular pathways followed by MHC molecules both before and after peptide association are critical to understanding T cell immunity. Using transient transfection of site-induced mutants of the invariant chain (Ii), we have found that the exon 3-encoded region of this protein is essential for binding to class II, for proper association and stability of class II α and β chains, for class II transport from endoplasmic reticulum to Golgi, and for inhibition of class II peptide binding. This region appears to play the same functional role as peptide plays for class I molecules, via occupancy of the class II binding groove while still a part of intact Ii. These data imply a modular structure-function relationship for invariant chain, and explain its contributions to early post-synthetic class II behavior.

Pulse-chase labelling, immunoprecipitation, and gradient density fractionation of B lymphoblasts show that class II and Ii enter early endosomes via the plasma membrane. Removal of Ii and peptide loading occur in multiple compartments that cofractionate with both late endosomes and early lysosomes. Thus, class II traffics to multiple endocytic compartments, consistent with a model in which class II acquisition of different protein determinants may occur in compartments of differing pH and hydrolytic capacity.

Intact extracellular proteins are usually not converted into peptides bound to MHC class I molecules, but some exceptions have been reported. To better define a possible exogenous to endogenous pathway of antigen presentation, we have established a model system in which phagocytic stimuli promote class I presentation of exogenous soluble protein. Our studies suggest that this does not occur due to induction of a special vesicular to cytoplasmic transport mechanism, but may instead reflect the rare breakdown of the phagosome membrane and entry of the antigen into the conventional cytoplasmic class I processing pathway. These data are of significance for vaccine design, and may also bear on CD8 T cells responses to some pathogens that normally reside in endocytic vesicles, but occasionally escape into the cytoplasm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00565-05 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Family of κB Regulators for Genes in the Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	D. Jiang	Graduate Student	LI, NIAID
	B. Combadiere	INSERM Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Regulation of genes for several lymphokines, as well as other molecules involved in the immune response, depend on a 10 bp DNA sequence termed, κB. This sequence binds a family of nuclear proteins, which are related to the mammalian Rel oncogene and the drosophila protein, dorsal, that control transcription of these genes. Importantly, the κB sequence is found in the human immunodeficiency virus (HIV). A cardinal feature of the κB sequence is that it permits transcription in a highly regulated fashion both temporally and in appropriate cell-types for specific genes. We are attempting to elucidate how this specific regulation occurs in T lymphocytes. We study NF-κB in nontransformed T cell clones following stimulation by antigen and antigen-presenting cells (APCs). Recently we have focused on comparing the regulation of various NF-κB subunits in the T_H1 and T_H2 subsets of CD4+ T lymphocytes. Evidence has suggested that there is a switch from the T_H1 to the T_H2 subset in the late course of AIDS. We have therefore studied the activation of the HIV long terminal repeat (HIV LTR) which is controlled by NF-κB. Our preliminary findings suggest that the HIV LTR is more active in T_H2 cells suggesting that viral production might be accelerated in the late phases of AIDS during the T_H1 to T_H2 switch. Further work will be directed at confirming and extending these findings and determining the molecular events responsible for this effect. We have also found that the microheterogeneity in DNA sequence among κB sites has regulatory significance. We have discovered a novel nuclear complex, termed NF-κC that interacts preferentially with a κB site in the interleukin-2 gene. The presence of NF-κC in a number of different biological conditions is inversely correlated with IL-2 gene expression in T cells. This suggests it may be a negative regulator. We have shown that NF-κC consists of a homodimeric complex of the NF-κB p50 subunit and that it can directly repress the function of the IL-2 promoter. Very significantly, this factor binds to the enhancer region of HIV. We postulate it may have a role in suppressing HIV viral transcription in resting T cells. Importantly, we have found that the binding activity of the NF-κC complex is governed by an inhibitory protein (IκC) that sequesters the NF-κC complex in the nucleus following antigen stimulation. Evidence currently suggests that the IκC inhibitor may be the proto-oncogene bcl-3, that is found is found at the breakpoint of chromosomal translocation in specific types of lymphomas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00566-05 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Regulatory Events in Establishing Mature T Cell Tolerance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	Lixin Zheng	Visiting Fellow	LI, NIAID
	K. Bruhn	Research Technician	LI, NIAID
	B. Combadiere	INSERM Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

LI, NIAID (Drs. William E. Paul and Keats Nelms)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

T cell tolerance has been found to occur in immature T cells in the thymus and also extrathymically in mature T cells. Our studies of mature T tolerance has revealed that they may undergo deletion or enter a functionally unresponsive state termed anergy. In anergy, a T-helper cell can be induced to "turn off" IL-2 production if it is stimulated through its T-cell receptor in the absence of costimulation. Previously we showed that regulatory proteins that govern IL-2 gene expression may be poorly activatable in response to antigen in anergic T cells. One important determinant of whether T cells will be normally activated by encountering an antigen/MHC complex is whether it is presented along with co-stimulatory stimulus. Co-stimulation is the signal provided by the interaction of surface molecules such as CD28 (on the T cell) and B7 or BB-1 (on the antigen-presenting cell). The presence of co-stimulation seems to be required for the normal T cell proliferative response to antigen. To understand the role of co-stimulation in IL-2 production, we have studied the effects of co-stimulation on various elements in the IL-2 promoter. Thus far these studies indicate that the transcriptional function of certain cis elements in the promoter can be augmented by co-stimulatory influences. Further studies are in progress of further define the molecular mechanisms involved. Finally, an important aspect of the success of an immune response to an invading micro-organism appears to be the specialization of the helper T cell response towards cells that either produce IL-2 (T_H1 cells) or IL-4 (T_H2 cells). We have studied the regulatory elements in the promoter of the IL-4 gene and detected a transcriptional trans-activator and its binding site (CS-1) that are restricted to and crucial for expression of T_H2 cells.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00622-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Measurement of Binding of Self and Antigenic Peptides to MHC Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Lisa Boyd	Chemist	LI, NIAID
	Marie Jelonek	Medical Staff Fellow	LI, NIAID
	Sergei Khilko	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LMB, NIDDK (E. A. Padlan); University of Adelaide, AU (J. McCluskey); LVD, NIAID (P. Gershon and B. Moss); LBS, LI, NIAID (S. Sadegh-Nasseri and R. Germain)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	4.2	0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our objectives in this project have been to examine the details of the interaction of self and antigenic peptides with the MHC class I molecule by detailed kinetic and equilibrium binding methods, and by three-dimensional computer modeling of structures of MHC/peptide complexes. Such studies permit us to examine underlying biochemical rules that govern the interaction of antigenic peptides with MHC molecules, which plays a central role in the initiation of the immune response. In addition, they provide a framework for understanding the larger question of how proteins bind small molecular weight peptide ligands.

Major accomplishments in the past year include the further development and application of quantitative kinetic assays for measurement of the interaction of peptides with purified MHC molecules in real time, and the extensive analysis of the binding of a larger number of peptides. In addition, methods have been devised for the quantitative emptying of MHC class I molecules and the retention of their peptide binding activity. In addition, general methods are being developed for the chemical coupling of engineered molecules tagged with histidine residues to metal-conjugated biosensor surfaces.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00623-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Folding and Assembly of MHC Molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David H. Margulies	Section Head	LI, NIAID
Others:	Shozo Sakuma	Guest Worker	LI, NIAID
	John Lam	Summer IRTA	LI, NIAID

COOPERATING UNITS *(if any)*

LICB, NCI (Randall K. Ribaudo); LB, NCI (M. Mage and L. Lee)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project has been to develop *in vitro* systems for the study of the assembly and folding of the MHC class I molecules. Past studies have focused on coupled *in vitro* transcription/translation systems for examination of the assembly of the three chains of this macromolecule: the class I heavy chain, β-2 microglobulin, and antigenic peptide. Recent results with *in vitro* mutants emphasize the value of such a system in evaluating parameters of assembly. Our current approach is to develop more biochemically defined methods, based on genetically engineered, bacterially produced single chain MHC class I proteins and synthetic antigenic peptides. We have demonstrated that biologically active, immunostimulatory MHC class I peptide complexes can be made by *in vitro* refolding of completely reduced and denatured molecules, suggesting that this is a feasible approach to produce large scale amounts of homogenous complexes for experimental use.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00624-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

New Chemical Tools for Immunology and Structural Biology Research

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John K. Inman	Section Chief	LI, NIAID
Others:	Patricia F. Highet	Technician	LI, NIAID

COOPERATING UNITS *(if any)*

Food & Drug Admin (B. Golding, H. Golding); State University, Utrecht, The Netherlands (H. Snippe); Lab. Pathology, NCI, NIH (H. C. Krutzsch, D. D. Roberts)

LAB/BRANCH

Laboratory of Immunology

SECTION

Bioorganic Chemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project covers the design, synthesis and use of new reagents and chemical constructs that will meet specific requirements in research projects of collaborating groups or individuals. Research goals fall within the fields of basic and applied immunology and structural biology.

For the preparation of conjugates and immunoadsorbents, heteroligation strategies have played a central role in our work. In these strategies, two disparate structures are cross-linked without self-polymerization by placing uniquely interacting functions on each component through chemical derivatization. We have favored the use of sulfhydryl and sulfhydryl-selective groups as the paired functions, with cross-linking occurring through formation of stable thioether linkages. New reagents have been developed for the placement of sulfhydryl-selective, haloacetyl (chloro-, bromo- and iodoacetyl) groups onto proteins, peptides and polymers. A US patent was issued on the synthesis and use of a novel reagent for placing bromoacetyl group as a side chain at any desired position of a peptide being synthesized by solid-phase or other sequential methods.

A new compound was designed and synthesized that serves as a lipid component in the preparation of liposomes and provides sulfhydryl-reactive iodoacetamido groups at their aqueous interfaces. Immunogenic liposomes were prepared by reacting the above material with synthetic peptide antigen epitopes containing a cysteine residue. Using peptides from the HIV-1 V3-loop, strongly immunogenic liposomes have been prepared that may serve as a prototype for an AIDS vaccine. The compound, a derivative of phosphatidylethanolamine, should be generally useful for incorporating biologically active peptides into liposome carrier constructs.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00625-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Phosphorylation in the Effector Functions of T-Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. V. Sitkovsky	Section Head	LI, NIAID
Others:	Mas Koshiba	Visiting Fellow	LI, NIAID
	S. Apasov	Visiting Associate	LI, NIAID
	V. Sverdlov	Visiting Fellow	LI, NIAID
	P. Smith	Research Associate	LI, NIAID
	Pearl Chen	Research Associate	LI, NIAID
	Pierpaola Correale	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5		5.5

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of our studies is to understand biochemical pathways and to identify key proteins (enzymes) that are involved in the activation and effector phases of important immune responses, such as T-cell-mediated cytotoxicity (toward virus-infected cells and toward tumor cells) and lymphokine secretion. It is expected, that this knowledge will enable us to selectively manipulate different functional responses of T-cells with specifically designed immunomodulators.

We identified a novel trans-cell ecto-phosphorylation/dephosphorylation system in lymphocytes, which supports a newly formulated general model of cell-cell interactions in the development and effector functions of lymphocytes. According to this model, receptor-ligand or cell-cell contact protein interactions are regulated by the state of phosphorylation of extracellular domains and by signal-transducing ATPo-receptors. We demonstrated the presence of, characterized, and purified a highly active and extracellularly located Casein II-like protein kinases (ectokinase) which we implicated in T-cell function. We also demonstrated the presence of a PPI class ecto-protein phosphatase. In addition, we cloned the cDNA coding for the T-cell's ATPo-receptor and obtained antibody to the extracellular domain of this receptor for future studies. We found that substrates of ecto-phosphorylation on the lymphocyte surface are either functionally important surface proteins themselves or are associated with them.

The cDNA cloning of these ecto-enzymes may have immediate immunopharmacological implications by allowing the targeting of novel surface molecules.

We continued studies of the role of cAMP-dependent protein kinase (PKA) by obtaining stably transfected cell lines and transgenic mice with a dominant-negative R1α subunit mutation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00626-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inhibitors of Protein Kinases (or Phosphatases) and Immunomodulators

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael V. Sitkovsky	Section Head	LI, NIAID
Others:	Pat Smith	Research Associate	LI, NIAID
	Sergey Apasov	Visiting Associate	LI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

Biochemistry and Immunopharmacology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	2

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our ongoing *in vivo* and *in vitro* studies of functionally important cellular populations and of the role of individual proteins in effector functions of T-lymphocytes have obvious immunopharmacological implications. Our studies identify the cell types and key proteins that should be targeted for efficient immunomodulation when there is a clinical need to inhibit or to enhance the intensity of the effector phase of the immune response. Our ongoing studies are concerned with finding most efficient inhibitors of enzymes we have implicated in lymphocyte functions. We found that it is possible to influence cellular responses of intact cells using peptides which mimic inhibitory domains of known protein kinases and have demonstrated that such peptides are able to block T-cell responses. The problem of peptide proteolysis due to ecto-peptidase activities is expected to be dealt with by the use of hydrophobic tail-modified peptides to improve intracellular intake. A novel set of molecular targets for immunomodulation is being explored in studies of ecto-protein kinases, ecto-phosphatases, and ecto-ATPase. The data suggest that after adequate characterization of these ecto-enzymes and the development of specific peptide inhibitors, it will be possible to take advantage of the extracellular location of this set of ecto-phosphorylation/dephosphorylation enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00627-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulatory Events in T Cell Development in the Thymus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael J. Lenardo	Senior Staff Fellow	LI, NIAID
Others:	L. Yin	Visiting Fellow	LI, NIAID
	J. Züñiga-Pflücker	Jane Coffin Childs Fellow	LI, NIAID
	Y.-N. Chang	Visiting Student, Cambridge Univ.	LI, NIAID
	D. Jiang	Grad. Student - George Wash. Univ.	LI, NIAID
	P. Schwartzberg	Leukemia Society Special Fellow	LI, NIAID

COOPERATING UNITS *(if any)*

Metabolism Branch, NCI (Dr. Louis Staudt); NCI (Dr. Harold Varmus)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our goal has been to define the important molecular events that control T cell maturation within the thymus. Within the thymus, hematopoietic precursor cells undergo a complex set of developmental events and emerge as mature T lymphocytes capable of specific antigen recognition. This developmental process appears to result from a series of signalling interactions between T cell precursors and a heterogeneous and as yet ill-defined set of stromal cells. In addition to T cells, a class of cytotoxic lymphocytes known as "natural killer" cells, which play potentially important roles in immune responses against cancerous or virally-infected cells, can also develop in the thymus. We are taking two approaches to thymic development at the molecular level. First, we have established a culture system in which day 14/15 triple negative fetal thymocytes can be stimulated to express early markers of thymocyte development including CD25, ICAM-1, and Ly-6A/E. We are studying the molecular signals that activate this early developmental step. During these studies we have discovered a novel precursor cell that gives rise to both T lymphocytes and natural killer cells. We have also obtained evidence for a novel developmental pathway that allows triple negative thymocytes to develop into double positive thymocytes. Second, we are creating genetically-engineered mice that are homozygous-deficient (knocked-out) for various genes that are highly expressed in thymocytes. We are currently focusing on three genes: i) Ly-GDI - a GDP-dissociation inhibitor protein that controls the activity of Rho, a member of the Ras family that may be involved in molecular signalling cascades; ii) Rlk - a newly described member of the btk/itk family of tyrosine kinases that have been recently implicated in inherited immunodeficiency states and are involved in signalling from surface antigen receptors; and iii) Ntk - a newly-described tyrosine kinase related to the Csk kinase that regulates various Src family kinases by phosphorylating inhibitory tyrosine residues. Since signalling interactions are critical for proper T cell differentiation, disruption of these signalling molecules may inhibit T cell development in ways that clarify the normal physiological roles of these molecules.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00628-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigen-Induced Apoptosis (Propriocidal Regulation) of Mature T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Michael J. Lenardo Senior Staff Fellow LI, NIAID

Others: Stefen Boehme, Postdoctoral Fellow, LI, NIAID; Lixin Zheng, Visiting Fellow, LI, NIAID; Hugh McFarland, CRADA Fellow, LI, NIAID; Jeffrey Critchfield, HHMI Scholar - UC-San Francisco, LI, NIAID; Matthew Freedman, HHMI Scholar - Univ Michigan, LI, NIAID; Galen Fisher, HHMI Scholar - Univ PA, LI, NIAID; Sonia Batra, Student - Harvard/Rhodes Scholar - Oxford, LI, NIAID; Josh Milner, Summer Student - MIT, LI, NIAID; Thomas Yoo, Summer Student - Pomona College, LI, NIAID

COOPERATING UNITS *(If any)*

Neuroimmunology Unit, NINDS (Dr. M. Racke, Dr. C. Pelfrey, Dr. H. McFarland); LI, NEI (Dr. R. Caspi); Dept Biotechnology, Univ Washington, Seattle (Dr. J. Goverman); Immunology Div., Alexion Pharmaceuticals (Dr. L. Matis)

LAB/BRANCH

Laboratory of Immunology

SECTION

Molecular Development of the Immune System Unit

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	3.5	1.5

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is based on our discovery that stimulation of the antigen receptor of mature T cells following exposure of the cells to mitogenic lymphokines such as IL-2 leads to the induction of programmed cell death or apoptosis (propriocidal regulation). This process can lead to the deletion of specific antigen-reactive T cells without affecting bystander cells. We are interested in three aspects of this effect: 1) the molecular events that take place, 2) whether this mechanism can explain certain phenomenon of immunological "suppression" that have been previously studied, and 3) the possibility of using this mechanism to pharmacologically induce the elimination of T cells involved in disease processes. Our results indicate that antigen-induced apoptosis can only occur at specific stages of the cell cycle. Further insights into the molecular mechanism of this effect may elucidate how CD+ T cells die in AIDS, which recent evidence has suggested occurs by programmed death. This may lead to therapeutics that block CD4+ cell loss in AIDS.

Several types of diseases arise from the over-stimulation of the immune system including: allergies, autoimmune disorders such as multiple sclerosis, myasthenia gravis, insulin-dependent diabetes, and rheumatoid arthritis, and tissue graft rejection. Activated T cells are the driving force behind the destructive immune responses in these diseases. Current therapies involve drugs such as steroids and cyclosporin A that impair, in a general way, T lymphocyte function and thus increase susceptibility to infections. We have discovered that the cytokine IL-2 causes T cells to die if they are subsequently stimulated through their antigen receptor. This previously unknown property of IL-2 should allow the specific elimination of certain receptor-bearing T cells which could form the basis for new clinical applications of IL-2. Our results thus far indicate that mature T cell deletion by the propriocidal mechanism can be useful for ameliorating disease in mouse models for multiple sclerosis and autoimmune eye disease. Other disease models are currently under investigation. This method for treatment of T cell mediated diseases should avoid the severe immunosuppression which is a side-effect of current therapies.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00630-03 LI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Cytokines in the Pathogenesis and Treatment of Autoimmune Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. M. Shevach	Section Head	LI, NIAID
Others:	A. Bonomo	Visiting Fellow	LI, NIAID
	B. Segal	Staff Fellow	LI, NIAID
	E. Payer	Special Volunteer	LI, NIAID
	L. Sakimura	HHMI, Scholar	LI, NIAID

COOPERATING UNITS *(if any)*

Neuroimmunology Branch, NINDS, NIH (M. Racke); Dept Neurology, Georgetown University School of Medicine (A. Pachner); Div Neuropathology, Albert Einstein College of Medicine (C. Raine)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

•(a) Human subjects •(b) Human tissues x •(c) Neither

•(a1) Minors

•(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ability of the immune system to discriminate between self and non-self is acquired in the thymus during T cell maturation. Self-reactive clones specific for antigens expressed intrathymically are eliminated inside that organ. T cells which can recognize self-antigens not represented intrathymically are not deleted and represent a potential danger. However, such cells are usually rendered tolerant when they recognize their target antigens in the periphery. Autoimmunity may be secondary to a failure of intra-thymic deletion of autoreactive T cells, to a failure to induce peripheral tolerance, or to a breaking of established immunological tolerance. We have concentrated our studies on an analysis of the pathogenesis of several different autoimmune states: 1) Thymectomy of 3 day old mice induces organ-specific autoimmune disease which is characterized by the presence of autoantibodies and T cell infiltrates in the affected organs. Lymph nodes of 3-4 day old mice contain a significant number of thymus-derived CD4+CD8+ T cells that are phenotypically immature, but are enriched in precursors of autoreactive cells. These cells exhibit enhanced reactivity in the syngeneic mixed leukocyte reaction and may be responsible for the initiation of autoimmune disease in the target organs. 2) To understand the factors that regulate immunologic tolerance, we have developed an in vivo model for the superantigen-specific priming of IL-4 producing (Th2) T cells by the injection of mice with superantigen and IL-4. High doses of superantigen were able to induce tolerance in both the naive precursors of IL-4 producing T cells and in T cells already primed for IL-4 production. These studies demonstrate that the IL-4 pathway is susceptible to tolerance induction in vivo and suggest that antigen-specific therapies to inhibit IL-4 production in allergic disease states are feasible. 3) Our ability to prime superantigen specific T cells in the presence of IL-4 raised the possibility that autoantigen specific T cells could also be primed for IL-4 production and could prevent the tissue damaging effects of autoreactive interferon-γ producing Th1 cells. The administration of IL-4 to animals with experimental allergic encephalomyelitis resulted in amelioration of clinical disease, the induction of antigen-specific IL-4 producing cells, diminished demyelination, and inhibition of the synthesis of inflammatory cytokines in the central nervous system. Modulation of an immune response from one dominated by excessive activity of Th1-like T cells to one dominated by the protective cytokines produced by Th2 T cells may have applicability to the therapy of certain human autoimmune diseases.

PHS 6040 (Rev. 5/92)

LABORATORY OF IMMUNOPATHOLOGY
1994 Annual Report
Table of Contents

ZO1-AI Project Number		Page
Summary		11-1
00013-30	Immunobiology and Pathogenesis of Virus Infection -- Lewis (Terminated)	11-3
00138-17	Viruses and Immune Response -- Morse	11-4
00284-13	Characterization of Pathogenic Murine Leukemia Viruses -- Hartley	11-5
00286-13	Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms -- Hartley	11-6
00465-09	Retrovirus-Induced Murine Immunodeficiency Syndrome -- Morse	11-7
00484-08	Mechanisms in Hematopoietic Cell Differentiation -- Holmes	11-8
00544-06	Molecular Basis of Pathogenesis of Murine Leukemia Virus -- Chattopadhyay	11-9
00692-02	Molecular Determinants of Murine Leukemia Virus Pathogenicity -- Torrey	11-10

Summary Statement
LABORATORY OF IMMUNOPATHOLOGY
National Institute of Allergy and Infectious Diseases
October 1, 1993 to September 30, 1994

The interests of this Laboratory are centered on various aspects of viral pathogenesis with particular emphasis on interactions of viruses with the immune system. This year marks the end of longterm outstanding studies of papovavirus pathogenesis from the Viral Oncology Section which has been headed by Dr. Andrew Lewis since the inception of LIP. Most of the work in the other sections of the Laboratory is concerned with retroviral pathogenesis while other studies focus on hematopoietic differentiation. The major accomplishments of the Laboratory in pursuit of these studies are as follows:

Retrovirus-induced immunodeficiency: Mouse AIDS (MAIDS) is a syndrome with many similarities to human AIDS that develops in mice infected with a mixture of murine leukemia viruses (MuLV) designated LP-BM5. A replication defective virus (BM5def) that encodes only a variant gag protein is the etiologic virus in the mixture. Mouse strains differ greatly in their susceptibility to MAIDS after LP-BM5 virus infection. These differences were shown to be due to polymorphisms of MHC class I and class II genes (Makino, Tang, Chattopadhyay, Fredrickson, Morse, Hartley) and to non-MHC loci (Hartley, Chattopadhyay, Morse). The role of class II genes in relation to disease susceptibility appears to be due to presentation of antigen, possibly superantigen, to CD4 T cells to stimulate rapid expansion and cytokine production (Giese, Morse). B cells may be the critical antigen-presenting cells in susceptible mice, but they are also critical for efficient expansion of BM5def expression and secondary infection of T cells and macrophages (Kim, Morse). Current studies indicate that conventional B cells are sufficient for induction of MAIDS although the presence of CD5 B cells may facilitate accelerated disease (Tang, Kim, Morse). Class I gene differences may act in concert with class II differences to limit both helper virus expansion and expansion of cells expressing BM5def (Hartley, Chattopadhyay, Morse). Studies of class I mutant mice also suggest, however, that cells responsive to class I molecules may have a detrimental effect on disease progression (Tang, Morse).

The contributions of cytokine expression to disease have been investigated using a series of gene knockout and transgenic mice as well as mice treated exogenously with cytokines or antibodies to cytokines. Previous studies suggesting that expression of Th2 cytokines - IL-4 and IL-10 - might be required for disease induction were conclusively negated by the finding that mice deficient in both IL-4 and IL-10 secondary to gene knockouts developed MAIDS with rapid onset (Morawetz, Giese, Hartley, Morse). Additional studies of mice deficient in IL-4 alone showed that they produced IgE in response to virus infection, even though IL-4 was considered essential for IgE production (Morawetz, Morse). IL-12 was shown to be effective in treating mice infected with LP-BM5 viruses. Treated mice had greatly reduced lymphoproliferation and their immune responses were significantly enhanced (Gazzinelli, Giese, Morse). The effect of IL-12 was found to be mediate by interferon gamma, as no

effects of treatment were observed in interferon gamma knockout mice (Giese, Morse). to determine if disease can be prevented, vaccinia virus vectors were produced that contain BM5def gag. They are being used to immunize mice and to provide targets for CTL responses (Kulkarni, Morse, Immuno Ag).

Lymphomas. Mice with MAIDS develop a at high frequency clonal populations of T and/or B cells that can be transferred to scid mice (Tang, Hartley Fredrickson, Morse). These tumors were studied for proviral integrations and were found to have alterations in Gfi-1 and Evi-5, two linked loci on chromosome 5 (Liao, Morse, Tang). Studies of NFS mice congenic for ecotropic virus induction loci permitted the development of a new classification system for mouse lymphomas based on the Kiel system for human lymphomas (Fredrickson, Hartley, Morse, Chattopadhyay, Lennert).

Hematopoietic differentiation. Stem cell precursors common to the T and B cell lineages have long been postulated but no conclusive evidence for their existence has been developed. Using adoptive transfer studies, it has been found that the monoclonal antibody LIP-6 defines a bone marrow subpopulation that is a precursor for B and myeloid cells but not for T cells (Holmes, Lantz, Taubenberger). This suggests that there are separate precursors for T and B cells.

Determinants of MuLV tropism. Moloney MuLV and a very similar virus isolated for a RF myeloid tumor are restricted for growth in Mus. dunni cells as compared to SC-1 cells. This difference was found to be associated with a single amino acid change in the envelope gene which may affect glycosylation of the protein or could affect the conformation and hydrophobicity of the molecule (Torrey, Hartley). Host range variants may be associated with distinct patterns of pathogenicity.

Studies of murine cytomegalovirus (MCMV). Mice infected with MCMV were found to undergo striking B cell activation with differentiation to Ig secretion occurring within a week of infection. The effect peaked at day 10 and resolved almost completely by two weeks post infection. Unexpectedly, this response to MCMV also occurred in mice lacking CD4 T cells (Karupiah, Hartley, Morse). The effect may be due to expression of interferon gamma by NK cells and possibly to other cytokines (Karupiah, Morse).

Administrative changes. Dr. Arun Kulkarni and Dr. Gunaseng Karupiah joined the Laboratory. Dr. Andrew Lewis, head of the Viral Oncology Section, retired from the Commissioned Corps after 31 years of service. Dr. Yvonne Eyler completed an IRTA fellowship in Dr. Lewis section. Ms. Catherine deRivera left her guest researcher position to go to graduate school. Ms. Nan Lofgren left the Laboratory Chief's secretary position to move to Building 1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00013-30 LIP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Andrew M. Lewis Jr., M.D., LIP
Others: Yvonne Eyler, LIP
David Alling, ODIR

COOPERATING UNITS *(if any)*

National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado (J.L. Cook); New York State Office of Mental Health.

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00138-17 LIP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Viruses and Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, M.D., Chief, LIP, NIAID

Others: Janet Hartley, LIP
Brian Murphy, LID
Arun Kulkarni, LID
Mark Connors, LID
Gunaseng Karupiah, LIP
Kanta Subbaro, LID

COOPERATING UNITS *(if any)*

Laboratory of Experimental Pathology (NCI), Division of Virology, CBER, FDA

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at understanding the mechanisms by which the immune system responds to viral infections. Responses by this system can be divided into components that occur within hours to several days after infection and do not involve antigen-specific responses and later components that involve highly specific responses by T cells and B cells. In many instances, the nonspecific and specific components of the response act synergistically to control infection, but in some cases viruses elicit responses that allow them to evade protective immunity and to persist in the host. Analyses of the early response to infection by several DNA viruses have demonstrated that production of nitric oxide (NO) by macrophages activated by interferon gamma can inhibit virus replication. The effects of endogenous NO can be mimicked by exposing virus-infected cultures to compounds that produce NO. These compounds have been found to be effective in controlling an RNA virus,influenza, as well as several DNA viruses. In other studies, we examined the response of mice to infection with mouse cytomegalovirus (MCMV). These studies showed that infection stimulated massive polyclonal B cell activation within 4 days of infection. The response peaked at day 10 and was over by day 14. Activation was associated with increased IgG levels probably secondary to high level expression of interferon gamma. This subversion of the immune system may permit early spread of the virus to the salivary gland where it can remain protected from immune elimination by the late occurring response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00284-13

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Pathogenic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Janet W. Hartley, Head, Viral Oncology Section, LIP, NIAID

Others: Herbert C. Morse, LIP
Torgny Fredrickson, NCI
Sisir Chattopadhyay, LIP
Yao Tang, LIP
Ted Torrey, LIP

COOPERATING UNITS *(if any)*

National Institute of Health in Japan, NCI-FCRDC, FDA, Laboratory of Neuroscience, NIDDK

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	1.3	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves the biological and molecular characterization of murine leukemia viruses (MuLV) with the aim of understanding host and viral factors involved in the pathogenesis of neoplastic and non-neoplastic sequelae of infection. A continuing major effort involves studies of the complex of viruses (LP-BM5) responsible for induction of murine AIDS (MAIDS), a condition characterized by progressive lymphoproliferation and immune system impairment; polyclonal lymphoid cell proliferation can progress to oligoclonal or clonal expansions of T and B cells. Disease induction is dependent on both B cells and $CD4^+$ T cells and on a defective MuLV (BM5def) whose only product is an altered gag protein encoded by p12 sequences. The presence of replication competent helper virus enhances disease induction markedly. The extent and kinetics of integration and expression of BM5def sequences differ in resistant and sensitive inbred mouse strains inoculated with helper-pseudotyped virus, with expression increasing and persisting in sensitive mice and generally being found not at all or only at early time points in resistant mice. When sensitive mice are inoculated with helper-free BM5def, the defective genome becomes established and persists in absence of any detectable disease and with variable expression. Infection with replication competent virus results in rapid development of MAIDS. In mice with advanced MAIDS oligoclonal B and T cell populations, detected by FACS analysis of cell surface antigens and by immunoglobulin or T cell receptor gene rearrangements, can frequently be transplanted to SCID mice to yield B and T cell lymphomas. Several T cell lymphomas have been found to display rearrangements of the preferred proviral integration site Gfi-1, The xid mutation, which results in altered B cell function, has been found to be correlated with early expression of ecotropic MuLV, often in high titer. In two mouse strains congenic for the mutation, B6.xid and BALB.xid, the viruses are B-tropic, indicating that the expressed virus carries a gag gene mutation not found in the wild type N-tropic virus normally isolated from young mice. In the B6.xid colony 2 populations of mice are present and in the sublines with high virus expression the B-tropic virus is transmitted epigenetically via milk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00286-13 LIP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Genetic Control of Murine Leukemia Viruses and Virus-Induced Neoplasms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Janet W. Hartley, Head, Viral Oncology Section, LIP, NIAID

Others: Herbert C. Morse, LIP
Torgny Fredrickson, NCI
Yao Tang, LIP
Ted Torrey, LIP
Sisir Chattopadhyay, LIP

COOPERATING UNITS *(if any)*

National Institute of Health in Japan, NIAID, NY State Dept of Health, Jackson Laboratory, Whitehead Institute, Northwestern University, FDA, Zentrum fur Pathologie, Kiel, Germany, McLaughlin Institute

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.9	1.8	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project covers studies aimed at expanding our understanding of the various genetically specified mechanisms involved in determining sensitivity and resistance of inbred mice to murine leukemia virus (MuLV) infection and disease induction. At present two major areas are under study, one involving exogenous infection of mice by the replication defective MAIDS MuLV as well as replication competent helper MuLVs, and the second studying the hematopoietic tumors developing spontaneously in NFS v-congenic mice - mice of similar virus negative, low spontaneous lymphoma background that carry germ-line integrations of ecotropic MuLVs derived from the high virus, high thymic lymphoma incidence AKR and C58 strains. In the first area of study, in which both Class I and Class II MHC effects on sensitivity and resistance to MAIDS have been described previously, the effects of unmapped genes outside the MHC complex that modify disease and may or may not affect helper virus replication are being studied. Having analyzed a number of strains for polymorphisms in disease induction and helper virus replication, we are now testing crosses of sensitive and resistant pairs [B10.RIII and RIIIS (both H-2^r), C57L and 129 (both $H2^b$), C57BL and 129 (both H-2^b), and SWXL-4 and SWR (H-2^q)] at various times post-inoculation for presence and degree of disease, replication of helper virus, and integration and mRNA expression of defective and helper virus. Attempts will be made to map non-H-2 genes affecting disease induction by use of microsatellite DNA PCR analysis. In the second area of effort, a large series of spontaneous v-congenic T and B cell lymphomas has been characterized for microscopic morphology, by immunological phenotyping, and for alterations in immunoglobulin and T cell receptor genes. In addition to relating individual tumors to lineage origin and clarifying the classification of mouse lymphomas, two B cell lymphomas - immunocytoma and marginal zone lymphoma - previously undescribed in mice have been characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00465-09 LIP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Retrovirus-Induced Murine Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert C. Morse III, Chief, LIP

Others: Janet W. Hartley, LIP
Sisir Chattopadhyay, LIP
Yao Tang, LIP
Nathalia Giese, LIP
WanKee Kim, LIP
Torgny Fredrickson, NC
R. Gazzinelli, LPD
F.A. Sher, LPD
L. Selvey, EIB, NCI
R. Hodes, EIB, NCI
A. Kulkarni, LIP
R. Morawetz, LIP

COOPERATING UNITS *(if any)*

Immuno AG; University Hospital, Geneva; Natl Inst of Health, Japan; DNAX; Genetics Inst; University of Cologne; Hoffman La Roche, Genentech.

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	3.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is designed to develop a fundamental understanding of the mechanisms by which retroviruses induce immunodeficiency. The central focus is on mice infected with the LP-BM5 mixture of murine leukemia viruses (MuLV) which causes progressive lymphoproliferation and severe immunodeficiency, a syndrome termed mouse AIDS (MAIDS) in some inbred strains. This virus mixture includes an etiologic replication defective virus (BM5def) that encodes a unique gag polyprotein only and can induce disease given on its own. Our efforts have been directed at: a) determining the cell types targeted for infection by BM5def; b) examining the consequences of infection with this virus in terms of cytokine expression and evaluating the contributions of cytokines to disease resistance or sensitivity; c) examining the possible therapeutic potentials of IL-12 treatment of infected mice and possible prevention of MAIDS by vaccination of mice with vaccinia recombinants carrying the BM5def gag gene. Our analyses showed that B cells are the immediate target for infection and the efficient infection of T cells and macrophages requires the presence of B cells. A crucial role for MHC class II antigens in MAIDS was revealed by the finding that no disease develops in mice incapable of expressing class II molecules. The pattern of cytokine expression in infected susceptible mice includes high level transcription of interferon gamma, IL-4 and IL-10. Use of mice deficient in expression of these cytokines demonstrated that IL-4 and IL-10 are not required for induction of MAIDS and that more disease develops in interferon gamma knockouts. Importantly, it was found that mice treated with IL-12, a cytokine that stimulates Th1 cells and interferon gamma expression, had a markedly beneficial effect on the course of MAIDS. Future studies will be directed at understanding how immunization or IL-12 treatment exert their positive effects on MAIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00484-04 LIP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms in Hematopoietic Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K. L. Holmes, LIP

Others: H.C. Morse, III, LIP
J.K. Taubenberger, LIP
G.F. Burton

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.0	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The LIP-6 monoclonal antibody identifies a novel protein expressed by mature B and myeloid lineage cells and by bone marrow pre-B cells. We have investigated whether LIP-6 can be used to isolate cells that are committed to B cell differentiation but lack the expression of CD45R(B220). The phenotypic identification of cells prior to CD45R(B220) expression would enable the study of mechanisms responsible for hematopoeitic cell lineage committment. A population of bone marrow cells has been identified that is LIP-6$^+$CD45R(B220)$^-$ Ly-5(CD45)$^{+++}$ that has the capacity to differentiate into B, T and myeloid lineages when inoculated into sublethally irradiated mice. This population was further divided phenotypically into L-selectin$^+$ and a L-selectin$^-$ populations, comprising 3-4% and 0.5%, respectively, of total bone marrow. In vivo reconstitution studies with these cells has shown that the L-selectin$^+$ cells differentiate only into B lineage cells but have a limited capacity for self renewal. In contrast, the L-selectin$^-$ cells were capable of differentiation within the B, T and myeloid lineages and probably contains pluripotent hematopoietic stem cells. Future experiments will determine the frequency of B cell progenitors within the L-selectin$^+$ cells as well as their other phenotypic and functional characteristics. LIP-6, in combination with CD43, L-selectin, and CD45R(B220), has also been used to delineate the terminal stages of B cell differentiation. LIP-6 expression is increased during the immunoblast, plasmablast and immature plasma cell stages, but is lost upon differentiation to the mature plasma cell. In addition, L-selectin$^-$ LIP-6$^+$ cells found in Peyer's patches and immunized lymph nodes correspond phenotypically to germinal center B cells. Experiments to verify this are in progress. In addition, attempts to isolate and clone the gene coding for the LIP-6 protein will continue. A B cell line, NFS-1.0, that expresses LIP-6 will be used to construct a cDNA expression library to be screened with the LIP-6 antibody.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00544-06

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis of Pathogenesis of Murine Leukemia Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others: H.C. Morse, LIP
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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have shown that a 4.9kb replication defective recombinant murine leukemia virus (MuLV), present in the LP-BM5 murine leukemia virus complex, is the proximal cause of a lymphoproliferative, immunodeficiency syndrome, murine AIDS (MAIDS). The defective virus (BM5def) was molecularly cloned and sequenced to reveal that the virus has a functional LTR and the gag gene is intact. Within the gag gene only the p15 and p12 are highly divergent from other MuLB. In studies of the role of gag protein(s) in pathogenicity we have found that although bacterially expressed p12 gag induced high levels of antibodies in both susceptible and resistant mice there was no induction of a protective immune response. In order to determine the consequences of constitutive expression we prepared 2 constructs of BM5def gag for use in establishing transgenic mice. One utilized SV40 promoter and immunoglobulin (Ig) heavy chain enhancer to direct the gag gene expression in B cells and although 1/8 founder mice expressed the transgene, it did not transmit through the germline. The second construct utilizes MHC class II (Ea) promoter, to allow expression in B cells, macrophages and dendritic cells. These studies suggest that high level expression of the defective virus may be toxic to cells and may provide a model for understanding adverse effects of retroviral genes on the immune system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AI 00692-02

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Determinants of Murine Leukemia Virus Pathogenicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project aims to elucidate the molecular basis for differences in murine leukemia virus (MuLV) in vitro infective host range, in the hope that the consequent knowledge will facilitate an understanding of differences in in vivo pathogenicity and the host elements responsible for restriction of retroviral replication. As a model system, we have been examining an ecotropic MuLV isolate (RF.AMLV) which, along with the ecotropic Moloney MuLV (MoMuLV eco), is greatly restricted for in vitro infection of a Mus dunni mouse fibroblast cell line. This is quite unusual, since the Mus dunni cell line is highly sensitive to infection by most other ecotropic MuLV. Serial passage of the RF.AMLV stock through the dunni cell line gave rise to a virus preparation with altered in vitro tropism. The molecular basis of this alteration was identified by DNA sequence analysis, which revealed that for over 2.1 kbp of the envelope (env) gene sequence, only one nucleotide base change distinguished the dunni cell-tropic preparation from the original virus stock. This mutation encodes a single amino acid difference in the envelope protein domain responsible for determination of MuLV tropism. Demonstration that this single amino acid change is sufficient to account for the observed changes in ecotropic host range was accomplished by cloning the mutant env sequence into a MoMuLV backbone; transfection of the resultant recombinant retrovirus gave rise to viral stocks exhibiting the mutant phenotype (i.e. altered ecotropic host range). Additionally, parental or mutant envelope proteins expressed from the same transfected cell type manifested the corresponding phenotype, eliminating any possibility that host cell determinants were solely responsible for the acquisition of the altered infectiousness. Analysis of similar ecotropic host range variants generated in the course of retrovirus infection in vivo is underway to ascertain whether similar or identical amino acid alterations are responsible for these examples as well. Structure/function analyses have been begun to elucidate the biochemical basis for the host range selectivity of the parental and mutant envelope protein-bearing viruses.

Laboratory of Immunoregulation
1994 Annual Report
Table of Contents

Z01 AI Project Number		Page
Summary		12-01
00700-01	Virologic and Immunologic Events Associated with Primary HIV Infection - Pantaleo	12-19
00676-02	Immunopathogenic Mechanisms of HIV Infection: The Role of Cellular Activation - Pantaleo	12-20
00585-05	Pathogenic Mechanisms in HIV and Other Retroviral Infections - D. Cohen	12-21
00677-02	The Role of Cytokines in the Regulation of HIV Expression - Kinter	12-22
00699-01	Cytokine Expression in HIV Disease - Graziosi	12-23
00678-02	The Role of Infection of Bone-Marrow and Thymus in the Pathogenesis of HIV Disease - Stanley	12-24
00703-01	Role of Dendritic Cells in HIV Pathogenesis - Weissman	12-25
00675-02	Studies of HIV-Infected Long-Term Non-Progressors - Fauci	12-26
00702-01	The Effect of HIV Infection on Gene Expression in $CD4^+$ T Lymphocytes - Bende	12-27
00587-05	Regulation of Gene Expression of the Human Immunodeficiency Virus - Dayton	12-28
00701-01	Effect of Therapy on HIV Viral Burden and Replication in Lymphoid Tissue - O. Cohen	12-29
00390-11	Anti-Retroviral Therapy of Human Immunodeficiency Virus Infection - Polis	12-30
00635-03	Clinical Trials for the Prevention and Treatment of HIV-associated Infections - Falloon	12-31
00636-03	Immunologic Approaches to the Therapy of HIV-1 Infection - Walker	12-32
00679-02	Gene Therapy for HIV Infection - Bressler	12-33

00634-03	Investigation of the SCID-Hu Mouse Model of HIV-1 Infection - Davey	12-34
00361-12	International Studies on the Acquired Immunodeficiency Syndrome - Quinn	12-35
00210-14	Immunoregulation of Human Lymphocyte Function - Kehrl	12-36
00431-10	A Molecular Biologic Approach to Immune Activation - Siebenlist	12-38
00213-14	Clinical, Immunopathogenic, Therapeutic Studies of Vasculitis and Other Diseases - Sneller	12-40
00358-11	Immunopathogenesis of *Chlamydia trachomatis* Infection - Quinn	12-41

Summary Report
Laboratory of Immunoregulation
October 1, 1993 through September 30, 1994

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation

Over the past several years, the Laboratory of Immunoregulation (LIR) has focused its research efforts on the delineation of the immunopathogenic mechanisms of human immunodeficiency virus (HIV) infection and disease. We have focused on the host factors involved in HIV disease and have developed a model of disease progression whereby the complex pathogenic mechanisms are multifactorial and multiphasic, and these phases overlap throughout the prolonged course of disease. It has become clear that only by appreciating in a comprehensive manner the complex immunopathogenic mechanisms of HIV disease can we form a solid scientific basis for the design of therapeutic and vaccine strategies. In this regard, we have focused our efforts on the various stages of HIV disease from acute primary infection, to prolonged clinical latency and ultimately advanced disease, and have studied the immunopathogenic mechanisms at the tissue, cellular, cytokine, and molecular levels. In addition, we have designed and executed therapeutic and vaccine protocols.

Virologic and Immunologic Events Associated with Primary HIV Infection

We had previously delineated the important role of lymphoid tissue in the pathogenesis of HIV disease by studying lymph node biopsies of infected individuals at various stages of disease from the early clinically latent stage through the advanced stage (see 1992-1993 LIR Annual Report). Despite the fact that a large proportion (up to 70%) of individuals with primary HIV infection experience a non-specific, mononucleosis-like clinical syndrome, hospitalization is required in only a minor percentage of individuals. Because of these logistic constraints, it has been difficult to obtain lymph node biopsies during acute infection in humans. Therefore, although in peripheral blood the virologic events (i.e., high levels of viremia and of HIV DNA and RNA synthesis in peripheral blood mononuclear cells [PBMC]) associated with primary HIV infection have been delineated, evidence for the initial localization of HIV in lymph nodes in humans is only indirect. In this regard, the simian immunodeficiency virus (SIV) animal model of acute infection represents an excellent experimental system to address this issue. The execution of sequential biopsies has demonstrated localization of SIV (i.e., numerous individual infected cells) in peripheral lymph nodes as early as 7 days post-inoculation. Trapping of virions in the follicular dendritic cell (FDC) network was detected by 2 weeks post-inoculation and increased over time, whereas the number of cells expressing SIV dramatically decreased. Trapping of virions coincides with the increase in the levels of antibodies efficiently binding complement. It is likely that similar events occur in humans. These studies indicate that the initial establishment of HIV infection occurs in the lymphoid organs. More importantly, viral particles already trapped in the FDC network during primary infection may be important for propagation of infection over time and represent a mechanism of virus escape from the immune response.

Vigorous HIV-specific cell-mediated and humoral immune responses are present during primary infection and certainly play a critical role in the downregulation of viremia. However, these immune responses are unable to clear HIV completely. Therefore, it is critical to delineate more precisely the nature of the primary immune response to HIV infection to determine the mechanisms responsible for the lack of complete efficacy of HIV-specific immune responses. To characterize the cell-mediated primary immune response to HIV infection, we have analyzed the T-cell receptor repertoire during primary HIV infection. In a longitudinal study of HIV-infected individuals following primary HIV infection, major expansions in a restricted set of Vβ families have been observed. The cells expressing the expanded Vβs were predominantly $CD8^+$ T lymphocytes and mediated HIV-specific cytotoxic activity against HIV envelope. Furthermore, nucleotide sequences of the recombinant clones of the expanded Vβs demonstrated the oligoclonal nature of these expansions. These results indicate that major oligoclonal expansions of $CD8^+$ T lymphocytes may represent an important component of the primary immune response to HIV infection. (Pantaleo, Graziosi, O Cohen, Muro-Cacho, Vaccarezza, Fauci, LIR/NIAID; Fox, Molecular Histolabs, Gaithersburg, MD; Orenstein, George Washington Univ; Baseler, PRI, Frederick, MD; Kotler, St. Luke's-Roosevelt Hospital, New York, NY; Shaw, Saag, Univ of Alabama/Birmingham)

The Role of Cellular Activation in the Immunopathogenesis of HIV Disease

We have investigated the occurrence of apoptosis *in vivo* in lymph node sections obtained from HIV-infected individuals at different stages of disease. In addition, the degree of apoptosis in HIV-infected lymph nodes was compared with that observed in lymph nodes obtained from HIV-negative individuals. Apoptosis has been readily detected in lymph nodes obtained from both HIV-negative and HIV-infected individuals. However, the degree of apoptosis in lymph nodes obtained from HIV-infected individuals was three to four times higher than that observed in the lymph nodes obtained from HIV-negative individuals; furthermore, in HIV-infected lymph nodes all functional compartments of the lymph node (i.e., cortex, paracortex, and sinuses) were extensively involved by this phenomenon. A significant correlation was observed between apoptosis, the degree of activation of the lymphoid tissue, and the chronic immune activation associated with HIV infection. In contrast, apoptosis intensity correlated neither with clinical stages of HIV disease nor with viral burden in lymph node. Finally, apoptosis was not restricted only to $CD4^+$ T cells; both B cells and $CD8^+$ T cells undergo apoptosis. Taken together, these results indicate that the increased intensity of the apoptotic phenomenon in HIV infection is caused by the general state of immune activation; however, it is independent of the progression of HIV disease and of the levels of viral load.

We have also investigated the effects of cyclosporin A (CsA) on HIV infection *in vitro* and in the SIV animal model of acute infection. CsA inhibits HIV infection *in vitro* at concentrations of drug easily tolerated in clinical practice. CsA cooperates with zidovudine (ZDV) in suppressing HIV infection *in vitro*. In acute SIV infection, CsA administration delays the onset of viremia, prevents the drop in $CD4^+$ T lymphocytes, does not increase the percentage of $CD8^+$ T lymphocytes, and potentiates antibody responses. Apoptosis intensity is reduced in lymph nodes of SIV-infected monkeys treated with CsA compared to that observed in lymph nodes of acutely infected untreated monkeys. These studies indicate that inhibition of the state of heightened immune activation in SIV/HIV infection may have a beneficial effect and may serve as the scientific basis for feasibility studies in HIV infection in humans. (Pantaleo, Muro-Cacho,

Vaccarezza, Fauci, LIR/NIAID; Orenstein, George Washington Univ; Fox, Molecular Histolabs, Gaithersburg, MD; Baseler, PRI, Frederick, MD; Martin, Tulane Univ; Dieffenbach, Black, Sager, DAIDS/NIAID)

The Mechanisms of Programmed Cell Death in HIV infection

By studying HIV-1 infection of normal T cells with primary clinical isolates, and by studying transfected T-cell tumor lines, we established that pathogenic, syncytium-inducing (SI) HIV-1 induces a specific form of programmed cell death (PCD) characterized by cell cycle arrest and cell death at the G2 checkpoint of the cell cycle. Antibodies to the G2/M cell cycle protein, cyclin B, identified individual cells dying of the cytopathic effect associated with HIV-1 infection. This process is now being studied in clinical material to attempt to learn what population of T cells undergoes this form of PCD, and to learn whether the percentage of affected cells can be used to predict progression to AIDS. The aberrant HIV-initiated form of PCD contrasted substantially with normal PCD occurring during T-cell development (negative selection) which usually occurs at the G1 point of the cell cycle. The phosphatase 2A inhibitor, okadaic acid, and the protein tyrosine kinase inhibitor, herbimycin A, were demonstrated to interfere with HIV-initiated cytopathicity. These differences between HIV-initiated PCD and negative selection offer encouragement for the development of therapeutic agents capable of specifically inhibiting HIV-mediated PCD. (D Cohen, Lane, Kolesnitchenko, Tian, Lempicki, Scully, LIR/NIAID; Samelson, CBMB/NICHD; Ashwell, BRMP/NCI; Shaw, Univ Alabama/Birmingham; Russell, McGowar, Scripps Inst; Hartmann, Cossman, Georgetown Univ; Hedrick, Newport, Univ California/San Diego)

The Role of Cytokines in the Regulation of HIV Expression

For several years the LIR has been investigating the role of cytokines in the regulation of HIV expression. The cellular and molecular pathways involved in the regulatory effects of proinflammatory and immunoregulatory cytokines on HIV expression/replication were further investigated this year. The complex interactions between various cytokines, such as interleukin (IL)-10 and tumor necrosis factor (TNF)-α or IL-6, and between cytokines and other cell-mediated signals, such as inducers of cellular differentiation, with regard to regulation of HIV expression were demonstrated in primary monocyte-derived macrophages (MDM) and/or in chronically infected cell lines. Investigation of the molecular basis for these effects demonstrated the multiple mechanisms, including transcriptional and post-transcriptional events, whereby cellular factors influence HIV expression. Autocrine/paracrine stimulation of HIV expression by endogenous cytokines was demonstrated in MDM and primary PBMC. In these systems, inhibition of cytokine secretion and/or activity by physiologic agents, such as IL-10, soluble cytokine receptors or antagonists, or by pharmacologic agents, such as pentoxifylline, resulted in significant suppression of HIV replication. Pentoxifylline was also tested *in vivo* in the SIV-infected macaque animal model. Although no inhibition of acute phase-related TNF-α production was observed, pentoxifylline-treated animals exhibited lower plasma and cell-associated viremia, lower apoptosis in the lymph node, and possibly delayed disease progression. Novel mechanisms of cell-mediated regulation of HIV expression/replication were demonstrated using PBMC or lymph node (LN) cells from HIV-infected individuals or chronically infected cell lines. IL-12 was found to allow isolation of HIV from unfractionated PBMC or LN cells in the presence of CD8-mediated viral

suppressive activity; preliminary data suggest that IL-12 can interfere with or bypass CD8-mediated suppression. Triggering of the CD30 molecule, a newly described member of the TNF receptor family, was found to potently induce HIV expression in chronically infected cells, most likely by activating the cellular transcription factor NF-κB. It is clear from these studies and studies previously conducted in our laboratory and other laboratories throughout the world that endogenous cytokines likely play a major role in the pathogenesis of HIV disease. Delineation of the scope and mechanisms of such effects should serve as the scientific basis for the design of therapeutic strategies. (Kinter, Fauci, Biswas, Weissman, Fox, Goletti, LIR/NIAID; Hirsch, LID/NIAID; Orenstein, George Washington Univ; Rabson, UMDNJ/RWJMS; Fox, Molecular Histolabs, Gaithersburg, MD)

Cytokine Expression in HIV Disease

We have previously demonstrated that endogenous cytokines play a major role in the regulation of HIV expression *in vitro* (see above). In addition, abnormalities of cytokine secretion have been reported in HIV disease. Since we had previously shown that the lymphoid organs are the major reservoirs for HIV burden and expression, we examined the constitutive and inducible expression of a panel of cytokines in LN and PBMC and purified lymphocyte subsets of HIV-infected individuals at various stages of disease. We found that the constitutive expression of cytokines was higher in both the PB and LN of HIV-infected individuals compared to uninfected individuals. This likely reflects the heightened state of immune activation in HIV infection. Of note is the fact that the HIV-inductive cytokines TNF-α and IL-6 were markedly over-expressed in the LN and BP of HIV-infected individuals and may contribute to the chronic state of virus expression. IL-1ß was also highly expressed in the PBMC of HIV-infected individuals. Despite the state of immune activation, IL-2 and IL-4 were poorly expressed in LN and PB of HIV-infected individuals. $CD8^+$ T cells, but not $CD4^+$ T cells, were responsible for the constitutive production of IL-10 and interferon-γ (IFN-γ). There was no shift in pattern of constitutive expression of cytokines in LN over the course of disease progression that would indicate a "switch" from a Th-1 to a Th-2 cytokine profile as determined by both cross-sectional analysis and longitudinal analysis of different patients and the same patients respectively over time. These studies shed insight into the cytokine milieu of the lymphoid tissue in HIV infection and should contribute to our understanding of the immunopathogenic mechanisms of HIV disease as well as provide potential targets for therapeutic intervention. (Graziosi, Pantaleo, Cohen, Fauci, LIR/NIAID; Sekaly, Fortin, IRCM, Montreal)

The Role of Infection of Lymphoid Microenvironment and Progenitor Cells in the Pathogenesis of HIV Disease

We have utilized the SCID-hu mouse model to study HIV infection of the human thymus. This tissue can be reproducibly infected with HIV with resultant thymocyte cell depletion and destruction of the thymic microenvironment. Thymocytes at multiple stages of maturation, including the single positive $CD8^+$ thymocytes, were found to be infected in this model; in addition, the thymic epithelial (TE) cells were infected. Infection of these latter cells does not seem to result in their death so that they may serve as an important tissue reservoir for HIV. The mechanisms of infection of both the $CD8^+$ single-positive thymocytes and the $CD4^-$ TE cells were investigated. Neither of these cell types is infectible *in vitro* with cell-free virus. Studies to

analyze infection after co-culture with HIV-infected cells are now underway. In addition, because the disruption of the thymic microenvironment is not paralled by direct infection of TE cells with HIV, studies to evaluate HIV-mediated disruption in cytokine and growth factor production and expression of important adhesion molecules have been initiated. The SCID-hu model has been useful in evaluating the role of apoptosis in HIV-induced cell death and preliminary studies indicate that it may provide a model for the study of superantigens in HIV infection. The role of cellular activation in HIV infection has been evaluated in this model. We have shown that pretreatment of SCID-hu mice with CsA, a drug that inhibits T-cell activation, inhibits or markedly decreases infection of the thymus with HIV. The mechanism of this inhibition has not been entirely elucidated but it appears that a decreased expression of activation markers does occur in the CsA-treated thymi.

Bone marrow studies are in progress to evaluate the role of the marrow as a reservoir for HIV and, in light of the findings regarding TE cells, to evaluate the role of stromal cell infection and disruption in the hematologic abnormalities that are common in HIV disease. Approaches to more complete reconstitution of the SCID mouse with a human immune system are being investigated and a transient circulating immune system has been observed after the simultaneous implantation in the mouse of human fetal thymus, liver, bone, intestine and lymph node. (Stanley, Fauci, Lee, Muro-Cacho, LIR/NIAID; Fox, Molecular Histolabs, Gaithersburg, MD; Orenstein, George Washington Univ; Baseler, PRI, Frederick, MD; Haynes, Duke Univ; Golway, ACB/NIAID)

The Role of Dendritic Cells in the Pathogenesis of HIV Infection

We have explored a model of early HIV infection where dendritic cells (DC) bind virus, transport it to lymphoid organs, and initiate active viral replication in $CD4^+$ T cells. Thus far, we have demonstrated that DC can bind HIV efficiently to their surfaces and when added to unstimulated T cells can induce a productive infection *in vitro.* This infection can be affected by the addition of exogenous cytokines and cytokine modulators that block endogenously produced cytokines. We have also identified three different populations of cells with dendritic morphology in peripheral blood. Two of these appear to be the same cells at different stages of differentiation, while the third does not express the DC marker HB15, does not stimulate in an autologous MLR, and is the only one infectible with HIV. Currently, animal model systems of early HIV infection are being developed to demonstrate that these *in vitro* observations may also occur *in vivo.* (Weissman, Li, Ananworanich, Fauci, LIR/NIAID; Baseler, PRI, Frederick, MD; Fox, Molecular Histolabs, Gaithersburg, MD; Tedder, Zhou, Harvard Univ; Orenstein, George Washington Univ)

Studies on HIV-Infected Long-Term Non-Progressors (LTNPs).

We have studied a group (n=11) of HIV-infected individuals whose disease has not progressed over periods ranging from 7 to 17 years. These individuals have been termed LTNPs in that they have been known to be HIV-infected and have been followed with serial (every 6 months) $CD4^+$ T-cell counts for at least 7 years. In addition, they have not received anti-retroviral therapy. To gain greater insight into potential immunopathogenic mechanisms of HIV disease, we studied these patients from a virologic and immunologic standpoint with particular emphasis on their lymphoid tissue. We had previously demonstrated (see 1992-1993 LIR Annual Report)

that lymphoid organs represent a major reservoir for HIV viral burden and are the primary sites for virus replication. In addition, we have previously demonstrated that lymphoid tissue (immune competent cells and lymph node microenvironment) is progressively destroyed as HIV disease progresses from early to advanced stages. In early stage disease, lymph nodes undergo hyperplasia and extracellular virions are trapped on the FDC of the activated and hyperplastic lymph node germinal centers. This is coincident with the partial clearance of viremia, although viral replication persists throughout all stages of HIV infection. As disease progresses (usually over years), the lymphoid tissue involutes, extracellular virus is no longer efficiently trapped, virus replication in individual cells increases, and plasma viremia increases. Using this model of disease progression as a framework, we studied our group of LTNPs. Levels of viral burden and virus replication were quite low in PB and LN MC of LTNPs compared to progressors. Of interest was the fact that relatively high levels of plasma viremia were noted in eight of 11 LTNPs, comparable to levels in HIV-infected individuals whose disease had progressed. The size of the LNs of LTNPs were generally smaller than those of progressors and the degree of follicular hyperplasia and the total nodal and germinal center areas were significantly less in LTNPs. Of note, and in striking contrast to progressors, the lymph node architecture of the LTNPs was preserved despite several years of infection. Variable degrees of virus trapping was detected; several patients had little if any trapping of extracellular virions. Virus particles were virtually never detected in tissue or cell suspensions by electron microscopy. Only rarely were individual cells detected that were expressing HIV. The lack of virus trapping could in part explain the fact that although only very few cells were actively producing virus in LN, plasma viremia seemed not to be efficiently cleared leading to relatively high levels of plasma viremia (see above). These data together with studies of lymphoid tissue in progressors suggests that disease progression is at least is part related to the deposition of virions on the FDC of LN germinal centers, persistent activation of LN, active virus replication, and progressive destruction of lymphoid tissue. From an immunological standpoint, HIV-specific cytotoxicity against gag proteins was consistently observed in PBMC of LTNPs. Proliferative responses to a variety of stimuli (mitogens, alloantigens, and recall antigens) were preserved in PBMC of LTNPs. Characterization of humoral immune responses is currently underway. Intensive study of LTNPs should shed valuable insight into the pathogenic mechanisms of HIV disease and hopefully will provide important information for the development of therapeutic and vaccine strategies. (Fauci, Pantaleo, Menzo, Vaccarezza, Muro-Cacho, Graziosi, Cohen, LIR/NIAID; Multicenter AIDS Cohort Study Group; Montefiori, Duke Univ; Orenstein, George Washington Univ; Fox, Molecular Histolabs, Gaithersburg, MD)

Regulation of Gene Expression of HIV

Recently we have focused on searching for proteins that specifically bind to the effector domain of Rev while it is bound to its RNA target sequence, the Rev response element (RRE). These are of interest because the effector domain targets cellular factors required for Rev activity to the Rev/RRE complex. We have now identified proteins that specifically bind the effector domain of Rev while it is bound to the RRE. These proteins are being excised from sodium dodecyl sulfate gels for sequencing and cloning. In related work we have proposed a model for the mechanism of action of the Rev protein of HIV-1 which holds that Rev cooperatively binds with cellular factors and the RRE to form a functional complex. Using cooperative binding as a criterion to search for Rev accessory factors we have detected in crude extracts a nuclear activity that promotes the binding of Rev to the RRE. This activity either cooperatively binds to the RRE

with Rev or catalyzes the binding of Rev to the RRE. Almost certainly the activity is due to the protein. Chromatographic analysis of the extracts has resolved at least four and possibly more separate activities. One of the activities is now about 20% pure. The physico-chemical data we have accumulated concerning the others will enable us to rapidly purify them. While we are cloning them, we will be studying their functions by microinjecting them (as semi-pure fractions) into mammalian cells along with appropriate indicator genes. In related work we have studied the interaction of the Rev protein with the RRE. We have found that RRE determinants strongly influence the binding of Rev molecules subsequent to the first binding event. This precludes models for Rev/RRE binding which hold that all of the specificity is in the first binding event and that subsequent multimerization of Rev along an RNA molecule involves only non-specific recognition of RNA by Rev protein. We have isolated a series of cellular genes regulated by HIV infection in macrophages. The upregulated genes include the endogenous human retrovirus-like element, Line-1, suggesting a possible evolutionary link between this endogenous virus and HIV. All upregulated genes will be evaluated for their suitability as therapeutic, immunologic targets. (Al Dayton, ET Dayton, Powell, Hus, Lee, Bende, Butini, Pantaleo, Zhang, LIR/NIAID; Maizel, Shapiro, LMB/NCI; Konings, Univ Colorado; Wingfield, Stahl, OD/NIH; Gallo, Klotman, LTCB/NCI; Baroudy, Kotwal, James N. Gamble Inst., Cincinnati, OH)

Effect of Therapy on HIV Viral Burden and Replication in Lymphoid Tissue

Lymphoid organs are the major *in vivo* reservoir of HIV-1. The effect of standard antiretroviral therapy on HIV burden (i.e., the frequency of HIV-infected cells) and expression was evaluated by sampling PB and lymphoid tissue of patients before and 8 weeks after either remaining untreated, remaining on ZDV, initiating ZDV, or adding didanosine (ddI) to ZDV. In individuals who did not undergo a change in therapy, patterns of histopathology, viral trapping, viral burden, and viral replication remained remarkably constant between week 0 and week 8. In the group that added ddI to ZDV therapy, modest treatment effects were seen in measures of viral replication; however, the viral burden in LN of these patients remained unchanged. The decrease in viral expression without change in viral burden implies that a relatively small population of newly infected cells accounts for the majority of active viral replication at any given point during the course of HIV disease. Similar studies are under way or planned to evaluate possible treatment effects of ZDV in later-stage patients. These include studies of dinitrochlorobenzene, an immunomodulator, in intermediate-to-late stage patients; and HIV Immunogen in early stage patients. (O Cohen, Pantaleo, Graziosi, Vaccarezza, Muro-Cacho, Fauci, LIR/NIAID; Orenstein, George Washington Univ; Fox, Molecular Histolabs, Gaithersburg, MD; Pavlakis, Schwartzentruber, FCRDC/NCI; Schnittman, Niu, DAIDS/NIAID; Holodniy, Palo Alto VA Medical Center; Loveless, Univ Oregon; Moss, Immunization Products, Carlsbad, CA)

Anti-Retroviral Therapy of HIV Infection

An intensive effort was directed toward examining the relationships between changes in viral burden, changes in viral genotype and phenotype, and changes in immunologic function in patients with HIV infection receiving a variety of anti-retroviral agents with different mechanisms of action alone or in combination. Viral isolates obtained from patients receiving ZDV alone were found to develop ZDV resistance sooner than those from patients receiving ZDV in combination with IFN-α. In addition, the emergence of resistance was associated with a precipitous increase

in the rate of CD4 decline. Neither ZDV nor IFN-α resistance were noted in patients receiving IFN-α alone. Studies of the nucleotide phosphonomethoxyethyl adenine (PMEA) and the immunotoxin CD4-Pseudomonas exotoxin (CD4-PE) were conducted for the first time in humans. Wild-type isolates were noted to have varying degrees of resistance to PMEA. PMEA resistance was mapped to amino acid 65 of the reverse transcription gene and found to confer cross-resistance to ddI. Although active *in vivo*, PMEA therapy was associated with hepatic and genitourinary tract toxicities. In contrast, no activity was seen in the context of CD4-PE administration. A randomized, controlled trial of the non-nucleoside L-697,661 demonstrated a dose-dependent development of resistance within 2 weeks of therapy that was due to a tyrosine to cysteine mutation at aa 181. New RNA-based methods were evaluated for the monitoring of viral burden. An infectious clone of HIV underwent an 8-base pair insertion in the region of the long terminal repeat. This mutant virus was replication competent and served as an excellent internal control for the measurement of particle-associated RNA in plasma. The branched-DNA (bDNA) technique was capable of quantitatively measuring changes in viral burden in large numbers of samples to a significantly greater degree than existing techniques. Changes in titers of plasma virus were found to fall within hours of initiation of anti-retroviral therapy and, utilizing a combination of polymerase chain reaction (PCR) and bDNA, responses to combination anti-retroviral therapy with ddI, ZDV, and the non-nucleoside inhibitor U-90,152 were clearly related to the pre-therapy presence of the 215 mutation of the RT gene. (Polis, Lane, Davey, Walker, Falloon, LIR/NIAID; Masur, Kovacs, CC/NIH; Urdea, Kolberg, Eastman, Chiron Corp; Salzman, Baseler, Natarayan, PRI, Frederick, MD)

Clinical Trials for the Prevention and Treatment of HIV-Associated Infection

An intensive effort directed at improving the prevention and treatment of acquired immunodeficiency syndrome (AIDS)-associated opportunistic infections continues. HPMPC, a nucleotide analog with activity against cytomegalovirus (CMV) was studied, and a safe and possibly effective dose was identified for Phase III trials. CMVIG-IV (Cytogam) was evaluated for possible activity for the prevention of CMV; it did not decrease the ability to culture CMV from body fluids nor did it prevent disease caused by CMV supporting the notion that antibody is of minimal value in the treatment of active CMV infection. A Phase I/II study of sparfloxacin, an investigational quinolone agent, and azithromycin, a new macrolide agent, for the treatment of infection caused by *M. avium* complex, was recently completed and data analysis is ongoing. A Phase I study of levofloxacin, an investigational quinolone agent that shows promise for the treatment of drug-resistant tuberculosis is almost complete. Enrollment in a study of IL-2 for the treatment of mycobacterial infection has begun. Studies of atovaquone, an agent with activity against *Pneumocystis carinii*, included completion of an assessment of the pharmacokinetics of the licensed tablet formulation of the drug and an assessment of the pharmacokinetics of an investigational suspension formulation of the drug; the suspension formulation was shown to be better absorbed than the tablet. In addition, a study of the efficacy and pharmacokinetics of the investigational suspension formulation of the drug for the treatment of pneumocystis pneumonia has just been completed, and data analysis is ongoing. The pharmacokinetics of dapsone and pyrimethamine were examined by using a population pharmacokinetic model. The assessment and follow-up of persons with idiopathic $CD4^+$ T-lymphocytopenia (ICL) is continuing. Finally, a new outreach effort for the enrollment of women and members of minority and under-served populations in clinical trials has begun, which includes seeing patients in a local community health

center. (Falloon, Lane, Davey, Polis, Walker, Sneller, LIR/NIAID; Masur, Kovacs, Spooner, Stearn, Baird, O'Neill, Kelly, Larson, Amantea, CC/NIH; Baseler, PRI, Frederick, MD; Turner, NCI)

Immunologic Approaches to the Therapy of HIV Disease

An intensive effort was directed toward studying the potential therapeutic aspects of immunologic approaches to HIV infection. A study evaluating the immunologic and antiviral effects of repeated administration of IL-2 and nucleoside analogs was continued. IL-2 therapy resulted in sustained increases in numbers of $CD4^+$ T cells and decreased expression of CD8 activation markers; the probability of manifesting these immunologic responses was shown to be directly associated with baseline CD4 count. Using a new plasma HIV RNA detection assay, transient and consistent increases in viral load at the end of each infusion were revealed. In follow-up of these findings, investigations into the mechanisms of IL-2 induction of HIV were begun. A study of IL-2 in patients with Kaposi sarcoma was initiated, in order to attempt to correlate immunologic and clinical responses. A randomized controlled trial comparing IL-2 plus nucleoside analogs to nucleosides alone was continued, and a randomized controlled multicenter trial comparing 3-, 4-, and 5-day infusions of IL-2 plus nucleosides to nucleosides alone was initiated. A dose escalation trial evaluating the safety and immunologic activity of subcutaneously administered IL-2 was initiated and an MTD was defined. Toxicities of IL-2 administered subcutaneously were identical to those seen with intravenous IL-2, but generally less severe. Adoptive immunotherapy using transfers of activated and expanded syngeneic polyclonal lymphocytes was completed. Marked but transient increases in CD4 counts were seen in the post-transfer period, often in association with increased viral replication in plasma. CD4 responses were directly related to baseline CD4 count. Studies with gene-marked cells were initiated as a prelude to attempts at gene therapy. A study generating random recombinatorial libraries of human immunoglobulin genes from HIV-infected individuals was continued. An anti-gp120 antibody was identified for clinical development. Studies evaluating the safety and activity of an anti-TNF-α antibody and a soluble TNF-α receptor were initiated. Inhibition of circulating TNF-α was not associated with short-term toxicity; no appreciable immunologic or virologic benefit was seen. (Walker, Fauci, Lane, Davey, Falloon, Polis, LIR/NIAID; Masur, Kovacs, CC/NIH; Schnittman, DAIDS/NIAID; Koenig, MedImmune, Gaithersburg, MD; Burton, Scripps Inst; Fyfe Chiron Corp; Blaese, NCHGR/NIH; Carter, Leitvin, CC/NIH)

Use of the SCID-hu-Peripheral Blood Lymphocyte (PBL) Mouse Model for the Study of Anti-Retroviral and Immune-Based Therapies of HIV Disease

In a series of several different experiments, 2-ß-Fluoro-dideoxyadenosine (FddA) was found to display markedly superior protection against infection relative to ZDV in the SCID-hu-PBL model of HIV-1 infection. The SCID-hu-PBL system was also used to examine the possibility of adoptive transfer of protective immunity using PBMC and concentrated anti-gp160 antibody harvested from gp160-immunized donors; however, no significant difference in the development of HIV-1 infection between the control and experimental groups was found. The SCID-hu-PBL model was then modified significantly to include direct reconstitution of SCID mice with peripheral blood lymphocytes from HIV-1-infected donors rather than through secondary infection by subsequent challenge with purified viral stocks. The rationale for this modification was to enable better simulation of natural infection by transferring critical elements of the human immune system

together with host-adapted virus specific for the donor. Intraperitoneal injection of harvested mononuclear cells from infected patients in this hu-HIV/PBL-SCID model led to successful engraftment in 84% of mice and caused no decrease in the number of human mononuclear cells harvested by peritoneal lavage relative to control mice engrafted with uninfected cells. Virus was readily recoverable in 98% of engrafted mice. Reconstituted hu-HIV/PBL-SCID mice that were untreated sustained a 75% decrease in human $CD4^+$ lymphocyte recovery in peritoneal wash relative to control mice, whereas concomitant treatment with FddA both significantly reduced $CD4^+$ cell depletion and inhibited viral recovery. Finally, whereas treatment with serial injections of human interleukin-2 or supplementation with high-titer anti-HIV human IgG had no effect upon either $CD4^+$ cell recovery or viral isolation, treatment with anti-TNF-α monoclonal antibody favorably influenced both of these parameters. These data support the utility of the hu-HIV/PBL-SCID model in studying antiretroviral and immune-based approaches to treatment of HIV-1 infection. (Davey, Boyle, Ruxrungtham, Lane, LIR/NIAID; Driscoll, Ford, DCT/NCI; Koenig, MedImmune, Gaithersburg, MD)

International Studies on the Acquired Immunodeficiency Syndrome

A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic, and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In Pune, India, we established a prospective cohort of over 2,000 high-risk individuals attending sexually transmitted disease (STD) clinics, of whom 25% were seropositive for HIV-1. Over a 4-year period, seroprevalence rose among commercial sex workers from 7.3% to 44%. Within the first year of follow-up, we documented an 11.6% seroincidence among men and women in this cohort. Phylogenetic analysis of viral strains isolated from these individuals indicate that both clade B and C strains are present with high-titer neutralizing antibody present against clade B isolates. Among patients attending a U.S.-inner city emergency department, HIV infection increased dramatically among young women, black males, and heterosexuals. Additional studies among STD patients in Baltimore demonstrated the low usage rate of condoms, and a high rate of recidivism for STDs and incident HIV infection. In perinatal studies of over 500 children born to HIV-infected women in Zaire, Haiti, Malawi, and the U.S., we documented a 28% perinatal transmission rate, which correlated with depressed maternal $CD4^+$ and elevated $CD8^+$ lymphocyte counts, anemia, p24 antigenemia, chorioamnionitis, and funisitis. In addition, perinatal transmission appeared to correlate with elevated concentrations of IgG-1 antibodies to the V3 loop in mothers, and with elevated levels of p24 antigen and secretory IgA to gp160 in cervical-vaginal secretions of transmitting mothers. Approximately 3% of infected children may have become infected postnatally from breastfeeding, and virologic studies demonstrated HIV DNA and p24 antigen in 50% to 70% of breast milk specimens within the first week following delivery. Diagnostic studies demonstrated a low sensitivity and specificity of HIV-specific IgA and PCR within the first week of life followed by increasing sensitivity of PCR by 1 month and IgA by 6 months. In studies of HTLV infection, we documented a 15.4% perinatal transmission rate of HTLV-I with an increased risk of transmission in women co-infected with HIV-1. In a prospective study of HIV and HTLV co-infection in Brazil, we documented an elevated $CD4^+$ lymphocyte count, β-2-microglobulin and p24 antigen levels among dually infected individuals. Further studies are planned to elucidate the immunologic modulations of HIV infection by co-infection with HTLV in these cohorts. (Quinn, Fauci, LIR/NIAID; Bollinger, Rompalo, Zenilman, Halsey, Kelen, Bartlett, Johns Hopkins Univ)

Immunoregulation of Human Lymphocyte Function

B lymphocytes are crucial cells in the host's response to infectious diseases. Our studies are directed at understanding the cellular and molecular mechanisms regulating B lymphocyte activation, proliferation, and differentiation. The cellular studies have been focused on identifying subsets of B cells and establishing culture conditions for the long-term growth of B lineage progenitors, immature B cells, and mature B cells. These studies should provide systems for examining gene expression in precise populations of B cells and for the analysis of genetic defects in B lymphocyte function. In addition, a panel of mAb against B-cell activation antigens has been made and their initial characterization completed. Several interesting and novel antigens have been identified. Molecular characterization is in progress.

We have continued our studies of several B lineage-specific proteins. We have determined that engagement of the B lineage-specific membrane protein CD22 activates an alternative B-cell activation pathway. This pathway is mediated by several tyrosine kinases, p72 *syk*, p53/p56 *lyn*, and p85 PI-3 kinase. These kinases have been shown to associate with the cytoplasmic portion of CD22 following CD22 cross-linking. Conversely, we have shown that the interaction of CD22 with its counter ligand on T cells provides a co-stimulation signal for T-cell proliferation. BL34 is a B-lineage protein localized in germinal center B cells. It has features suggestive of a transcription factor. We have found that the BL34 protein is a member of an expanding gene family. A cDNA clone for the murine equivalent of BL34 has been isolated and will be used to identify a murine genomic clone for gene targeting experiments. The murine cDNA has been used to create a transgenic mouse where BL34 expression has been targeted to κ light chain-bearing B cells using a κ gene promoter and enhancer. One such mouse has been obtained and is currently being bred to create a BL34 transgenic mouse line. A new member of the BL34 family has been isolated from activated B cells and has been denoted OL1. OL1, in contrast to BL34, has a more widespread tissue distribution and is a larger protein. Another B lymphocyte membrane protein that we have studied is encoded by the BL11 gene. A fusion protein between the cytoplasmic portion of IgG and the extracytoplasmic portion of BL11 has been produced. Studies attempting to identify a ligand for BL11 are in progress. Our studies of the GC kinase gene have continued. GC kinase-specific antibodies have been made. Using the antibodies we have shown that it is capable of auto-phosphorylation and that the protein is localized predominantly in germinal center B cells. A search for *in vivo* substrates is in progress. In collaboration with Dr. J. O'Shea we have shown that Jak-3, a recently discovered member of the Jak kinase family, identifies a unique subset of B cells.

The mechanisms of B lineage-specific gene expression of the CD19, CD20, CD22, BL34, and Bruton's tyrosine kinase (BTK) genes are under investigation. Several transcription factors have been identified to be important in the regulation of these genes including BSAP, Oct-2, and ETS family members. *In vivo* footprinting has been used to identify a number of important cis-elements including several elements that appear to bind novel DNA binding proteins. Mutant B-cell lines with markedly reduced levels of CD20 (two cell lines) and CD19 (10 cell lines) have been isolated. A B-cell line derived from a patient with Bruton's gammaglobulemia, which is deficient in BTK mRNA, has been obtained from Dr. M. E. Conley. Interestingly, these mutant cell lines apparently have a specific defect in the expression of only one gene suggesting that

each gene is differentially regulated. These studies should lead to a better understanding of the cis-elements and transcriptional factors important in B lineage specificity and in the control of B-cell differentiation.

Two human homeobox genes, HLX and HB9, first identified in activated B cells have been found to be expressed at high levels in hematopoietic progenitors. In addition, the HB9 gene has been found at high levels in pancreas and certain colonic tumors. The HB9 and HLX coding sequences have been introduced into retroviral vectors in order to dysregulate their expression in hematopoietic progenitors. Both the human HLX and HB9 genes have been isolated and the nucleotide sequence of the loci determined. The HLX and HB9 promoters have been identified and found to contain a number of interesting motifs. HLX promoter-CAT constructs have been made and found to be very active in the HLX-positive cell line K562. Murine genomic clones have been isolated and targeting constructs for each of these genes are being made.

These studies are resulting in a better understanding of normal B-lymphocyte function. As our knowledge of B cells increases we should gain insights in some of those diseases characterized by abnormal B-cell function and be able to develop methodology for the manipulation of B-cell responses (Druey, Himmelman, Kang, Riva, Shaz, Thevenin, Kehrl LIR/NIAID; Coligan, BRB/NIAID; Najfeld, Mount Sinai Medical Center, New York, NY; Katz, Georgetown; Tedder, Harvard Univ; Deguchi, Toyoma Univ, Japan; Rieckmann, Neurologische Klinik und Poliklinik, Germany; O'Shea, PRI, Frederick, MD)

Mechanisms Underlying Immune Responses: Activation of NF-κB

NF-κB is an essential regulator of many genes whose encoded functions are critical for immune and inflammatory responses, acute phase responses, and other defensive cellular reactions. This transcription factor also plays a prominent role in the regulated expression of many viruses, including HIV. NF-κB is a family of distinct dimeric factors, all composed of members of the Rel/NF-κB family of polypeptides. NF-κB lies dormant in the cytoplasm of cells, complexed with its inhibitor IκB-α. Activation signals related to or derived from pathogenic conditions cause rapid translocation of NF-κB into the nucleus due to the inactivation of the IκB-α inhibitor.

We have begun a systematic investigation into the molecular mechanisms by which IκB-α is inactivated by extracellular signals. We have demonstrated that activation of NF-κB proceeds via phosphorylation of IκB-α, followed by proteolytic degradation of the inhibitor. Activation leads to the transient appearance of a slower-migrating phosphorylated form of IκB-α. We have also determined that activation of NF-κB does not involve a phosphorylation-induced release of IκB-α from NF-κB as originally thought. Rather, phosphorylation of IκB-α appears to only mark IκB-α for proteolytic attack. IκB is then degraded while still bound to NF-κB. Consequently, proteolysis is essential for NF-κB activation. This conclusion is also supported by experiments in which the protease inhibitor calpain completely blocks NF-κB activation and IκB-α degradation while permitting phosphorylation of IκB-α to proceed. Proteases may therefore be therapeutic targets to prevent the induction of inflammatory responses by blocking NF-κB activation.

We have developed a model system to discover the molecular mechanisms underlying the activation of NF-κB by the HTLV-I Tax protein. HTLV-I immortalizes human T cells by

activating transcription factors like NF-κB through Tax. In our model system Tax is inducible by zinc in Jurkat cells permanently transfected with a metallothionein promoter-driven Tax expression vector. We have found evidence for two distinct ways in which Tax may activate NF-κB. Tax appears to induce proteolysis of IκB-α. However, because activated NF-κB immediately reinduces IκB-α, the steady-state level of IκB-α in the presence or absence of Tax does not change. Nonetheless, the significantly increased turnover of IκB-α may weaken its effectiveness in inhibiting NF-κB complexes, allowing for some NF-κB to escape to the nucleus. A second mechanism by which Tax activates NF-κB relates to its ability to bind the p100 protein, the precursor for p52, which can function like an IκB-α inhibitor. Tax antagonizes the ability of p100 to retain NF-κB in the cytoplasm, freeing NF-κB to translocate to the nucleus. At least some of the cytoplasmic pools of NF-κB are tied up in such complexes independent of IκB-α. Both Tax-induced mechanisms may be required to cause substantial activation of NF-κB.

We have uncovered the molecular explanation for an unusual NF-κB complex in sublines of U937 cells that do not support the growth of HIV. Seprocidins (a special class of serine proteases) are released from granules during cellular extraction enabling them to specifically process the p65 subunit of NF-κB *in vitro.* The resulting NF-κB complex has an altered mobility in gel shift experiments. These proteases are present only in those sublines that do not allow HIV to replicate well. It is possible that these proteases have a negative effect on HIV replication, most probably during the initial infection phase. (Siebenlist, Brown, Franzoso, Kanno, Lin, Tomita-Yamaguchi, Biswas, Poli, Bressler, Fauci, LIR/NIAID; Kelly LP/NCI; Woychik, Oak Ridge National Laboratory; Golding, FDA; Ozato, LMDI/NCI)

Clinical, Immunopathogenesis, and Therapeutic Studies of Vasculitis and Other Related Diseases

We have continued our study of the efficacy and safety of low-dose methotrexate (MTX) in treating Wegener granulomatosis (WG). Forty-two patients who did not have immediately life-threatening disease have been entered into the study to date. Glomerulonephritis was present in 21/42 (50%) of patients. The median follow-up was 23.4 months (range 1.2 to 45.6 months). Weekly administration of MTX and prednisone resulted in remission of disease in 30/42 patients (71%). The median time to remission was 4.2 months. Twelve patients did not achieve remission: three patients had progressive disease that required institution of cyclophosphamide (CP) therapy; five patients had clinical improvement but continued to exhibit signs of active disease; two patients developed MTX-induced pneumonitis prior to achieving remission; and two patients died of opportunistic infections prior to achieving remission. For the 30 patients who achieved remission: 23% relapsed within 1 year of achieving remission and 40% relapsed within 2 years. The estimated median time to relapse for all patients achieving remission was 29 months. In patients with glomerulonephritis at study entry, relapse occurred earlier (35% by 1 year) than in patients without glomerulonephritis (no relapses at 1 year); however, after 2.5 years the estimated percent of patients who had relapsed was similar in the two groups (50% vs 57%; p=0.1). Eight patients who relapsed were treated with a second course of MTX plus prednisone. A second remission was induced in 6/8 (75%) patients. MTX plus prednisone may be an acceptable alternative form of therapy for selected patients with WG. In preliminary studies we have used skin blisters induced by suction as a method to study the *in vivo* inflammatory response of patients with WG. We have shown that patients with active WG produce 50- to 100-fold higher levels of TNF-α in blister fluid when compared with normal controls. Such high TNF-α levels are not seen in blister fluid from patients with a variety of other inflammatory and infectious

diseases. Levels of IL-1ß and IL-6 were also significantly elevated in blister fluid from patients with active WG. These findings have important therapeutic implications since specific inhibitors of TNF-α and other pro-inflammatory cytokines are currently available and may be effective in the treatment of WG and related systemic vasculitides. (Sneller, Kerr, Talar-Williams, Fauci, LIR/NIAID; Travis, NCI; Gallin, Hallahan, OSD/NIAID)

Immunopathogenesis of *Chlamydia trachomatis* Infections

Over the past decade studies have been in progress to define the clinical spectrum and transmission efficiency of *Chlamydia trachomatis* infection, to develop improved molecular amplification assays for diagnostic purposes, and to examine the pathogenesis of *C. trachomatis* and *C. pneumoniae* infection in experimental animal models and in patients with genital, ocular, respiratory, and cardiac disease. Following the development of PCR for *C. trachomatis* in our laboratory, we have been able to demonstrate a marked increase in sensitivity of detection of chlamydial genital and ocular infections in patients attending STD clinics and in trachoma-endemic areas, respectively. In studies of over 4,000 patients, sensitivity and specificity of PCR for *C. trachomatis* was 91% and 99%, respectively, for endocervical, urethral, and urine specimens compared to culture, which ranged from 60 to 75%. The ability to screen urine from both men and women by PCR provides a non-invasive technique for the routine screening of this common STD. Utilizing this technique in adolescent school-based clinics, we demonstrated a seroprevalence of nearly 30%. Applying molecular amplification in transmission of *C. trachomatis*, we have been able to demonstrate an equal bi-directional transmission efficiency rate of 68% for *C. trachomatis* in contrast to previous studies, which demonstrated lower transmission rates by culture. Sequence analysis of PCR-amplified DNA demonstrated the same sequence in paired sexual partners, illustrating the accuracy and validity of PCR. Similar results have been observed utilizing a new molecular assay called ligase chain reaction. This assay demonstrated extremely high sensitivity and remarkable specificity with genital and urine specimens from both men and women for both *C. trachomatis* and *Neisseria gonorrhoeae*. We also developed a new serologic technique for the measurement of antibodies to the major outer membrane protein (MOMP) of *C. trachomatis.* This transcription-translation radioimmunoassay may help to further analyze the immune response to MOMP and other chlamydial antigens during vaccine trials. In the evaluation of *C. pneumoniae* infections, we demonstrated *C. pneumoniae* infection in 8% of patients with respiratory disease and in no asymptomatic healthy controls. *In vitro* antimicrobial susceptibility studies demonstrated superior efficacy of azithromycin and clarithromycin compared to erythromycin and tetracycline. Finally, we have been able to propagate *C. pneumoniae* in pulmonary alveolar macrophages, human aortic endothelial cells, human umbilical vein endothelial cells, and in aortic artery smooth muscle cells, providing support to the hypothesis of an association of *C. pneumoniae* with the development of atheromatous plaques in individuals with coronary heart disease. These observations indicate that more studies of this relationship are warranted (Quinn, LIR/NIAID; Gaydos, Viscidi, West, Zenilman, Rompalo, Johns Hopkins Univ)

Honors, Awards, and Scientific Recognition

During the past year, members of the LIR have received a number of awards and honors. Dr. Fauci continued to serve on a number of committees of scientific and administrative importance such as the Institute of Medicine Roundtable for the Development of Drugs and Vaccines against AIDS. He is also Councillor in the Association of American Physicians.

Dr. Fauci serves on the Editorial Boards of *The New England Journal of Medicine, The Journal of Immunopharmacology, Journal of Biomedical Science, Clinics in Immunology and Allergy, EOS, Cellular Immunology, The Journal of Molecular and Cellular Immunology, AIDS Research and Human Retroviruses,* and *AIDS Patient Care.* He is also an advisory editor of *The Journal of Experimental Medicine.* He serves on the Advisory Boards of *Immunopharmacology and Immunotoxicology, Clinical Immunology and Immunopathology,* and the *Journal of Clinical Immunology*, and he is on the Editorial Advisory Council of *La Ricerca in Clinica e in Laboratorio.* He continued his duties as associate editor of the *Journal of the Acquired Immunodeficiency Syndrome.*

Dr. Fauci continues as editor of "Harrison's Principles of Internal Medicine," "Transactions of the Association of American Physicians," "Current Therapy in Allergy, Immunology and Rheumatology," and "Harrison's Principles of Internal Medicine - Companion Handbook." He is an associate editor of "Current Therapy in Internal Medicine" and "AIDS, Etiology, Diagnosis, Treatment, and Prevention." In addition, he is an editor along with Dr. John I. Gallin of "Advances in Host Defense Mechanisms."

Dr. Fauci received Doctor of Sciences, Honoris Causa, degrees from the State University of New York at Farmingdale and The University of Connecticut Health Center.

This past year, Dr. Fauci delivered the prestigious Cartwright Lecture of Columbia University College of Physicians and Surgeons, and the First Annual Linda Laubenstein, M.D. Memorial Lecture at The New York University Medical Center. He delivered a state of the art lecture at The First National Conference on Human Retroviruses and Related Infections as well as lectures at the Colloquim of "Cent Gardes," in Marnes-la-Coquette, France, the College of the Holy Cross Sesquicentennial Alumni Lecture Series, and the Ninth International Conference on AIDS in Berlin.

Dr. Fauci was the keynote speaker at the Convention of the American Medical Student Association and the Biotech '94 Symposium on AIDS in Florence, Italy.

Dr. Fauci was an invited symposium speaker at the National Academy of Sciences Colloquium on Changes in Human Ecology and Behavior: Effects on Infectious Diseases; the annual meeting of the American Society for Cell Biology; a symposium in honor of Bernard Fields at Davis, California; the Sesquicentennial Scientific and Educational Symposium at Case Western Reserve School of Medicine; and the Joint Symposium on AIDS of the Interscience Conference on Antimicrobial Agents and Chemotherapy/Infectious Disease Society of America. Dr. Fauci was an invited speaker at the Nobel Conference on Cytokines in the Pathogenesis and Treatment of Infectious Diseases in Stockholm, the AIDS and Public Debate Conference of the AIDS History Society and the American Association for the History of Medicine, the Howard Hughes Medical

Institute Workshop on the Molecular Biology of the Human Immunodeficiency Virus, and the AIDS Action Foundation Conference on HIV Prevention Vaccines.

In addition, he delivered the commencement address at University of Connecticut Health Center.

Dr. Fauci received the following awards: The Cartwright Prize of Columbia University College of Physicians and Surgeons, the Humanitarian Award of the Tiro a Segno Foundation, and the Commission of Honor Award of the State University of New York at Farmingdale.

Dr. H. Clifford Lane was promoted to the rank of Professorial Lecturer in Medicine, George Washington University School of Medicine. He was also elected to the American Society for Clinical Investigation, and he won the Distinguished Service Medal, USPHS.

Dr. Thomas Quinn served on the Institute of Medicine Expert Panel on "Sexually Transmitted Diseases Control Efforts in the U.S. and Their Role in HIV Prevention;" Institute of Medicine Expert Panel on "Changing Demography of HIV epidemiology: Implications for Clinical Research;" National Academy of Science Panel on "Data and Research Priorities for Arresting AIDS in Sub-Saharan Africa;" Office of AIDS Research Working Group on International Scientific Research Cooperation and Strategies; Office of AIDS Research Coordinating Committee on Natural History and Epidemiology for the FY 96 NIH Plan for HIV-Related Research.

Dr. Quinn also serves as a member of the FDA Anti-viral Advisory Committee; as a member of the U.S. Japan Panel on AIDS and Related Retroviruses; and he participated in the U.S.-Mexican Biomedical Research Development Workshop. He served on the Advisory Council for the Women's Health Coalition and Campaign for Women's Health. Dr. Quinn was an invited speaker at the National Academy of Sciences Colloquium entitled "Changes in Human Ecology and Behavior: Effects on Infectious Diseases" presenting the paper, "Population Migration and the Spread of Types I and II Human Immunodeficiency Viruses" (PNAS 1994;90:2407-14). He was also a plenary speaker at the Tenth International Meeting of the International Society for STD Research in Helsinki, Finland, and at the Eighth International Symposium on Human Chlamydial Infection in Chantilly, France. He was Chairman of the Symposium on Perinatal Transmission at the First National AIDS Conference in Washington, DC, and was the keynote speaker at the University of Pittsburgh School of Public Health on World AIDS Day. He was Visiting Professor at the University of Washington School of Medicine and at Cornell University School of Medicine. Dr. Quinn continues to serve on the Editorial Board of *AIDS Research and Human Retroviruses*, *Infectious Diseases in Clinical Practice*, *International Journal of STD and AIDS*, *Journal of the Acquired Immunodeficiency Syndromes*, *Journal of Infectious Diseases*, *Journal of Women's Health*, *Sexually Transmitted Diseases*, and *AIDS*. He received an honorary award from Wilkes College and the Luzerne County Medical Society. In addition, one of his articles entitled, "Risk for Perinatal HIV-1 Transmission According to Maternal Immunologic, Virologic and Placental Factors" was awarded the 1994 Charles C. Shepherd Award (JAMA 1993;269:2853-59).

Dr. Ulrich Siebenlist was an invited speaker at the New York Academy of Science Symposium; NIH Research Day; the I.M.P. Annual conference in Vienna, Austria; and the Gordon Conference in Cancer. He also won an NIH Merit Award.

Dr. Giuseppe Pantaleo won the 1993 NIH Award of Merit. In addition, he was a keynote speaker at the following: VII Italian National Meeting on AIDS, Bari; Pathogenesis of AIDS - Medical Research Council Workshop, London; 50th Annual Meeting of American Academy of Allergy and Immunology; 8th annual meeting of the Canadian Society for Immunology, Montreal; 3rd International Conference on Cytokines, Florence; Biotech 94, Florence; 4th Annual Clinical Care Options for HIV Disease Symposium, Phoenix; Gladstone Institute of Virology and Immunology, San Francisco; Chairman, Workshop on Immunopathology of HIV Infection, European Immunology Meeting, Barcelona.

Dr. Sharilyn K. Stanley won the 1993 USPHS Commendation Medal.

Dr. David Cohen was a plenary speaker at three national conferences on HIV pathogenesis, and at an international symposium on programmed cell death sponsored by the National Institutes of Health.

Dr. Michael Polis won the Meritorious Achievement Award from the Infectious Disease Society of America.

Dr. Judith Falloon was elected Secretary-Treasurer of the Greater Washington Infectious Diseases Society. She also won an NIH Merit award and the James H. Nakano Citation from the CDC for her work on idiopathic $CD4^+$ T-lymphocytopenia.

Dr. Priscilla Biswas won the 1993 CIS Science Recognition Award for New Investigators.

Dr. Claire Thevenin received the Henry Christian award from the Merck Corporation for the outstanding abstract in gene regulation at the 1994 American Foundation for Clinical Research/American Society for Clinical Investigation/Association of American Physicians meeting in Baltimore, MD.

Administrative, Organization, and Other Changes

The major theme of the LIR continues to be focused on studies of the immunopathogenesis and therapy of human immunodeficiency virus (HIV) disease and the acquired immunodeficiency syndrome (AIDS). In addition, the laboratory studies the cellular and molecular mechanisms of the regulation of the human immune system in normal and disease states other than HIV disease. In keeping with the downsizing of the Intramural Research Program NIH-wide and the mandated decrease in full-time equivalents (FTEs), the LIR has been decreased by several FTEs for the coming fiscal year.

Two new Sections have been created within the LIR with no addition of resources. The B Cell Molecular Immunology Section was established and Dr. John Kehrl has been appointed as Section Head. The Immune Activation Section has been established and Dr. Ulrich Siebenlist has been appointed Section Head.

Dr. Guido Poli left the LIR after 8 years to return to Milano as Chief of the AIDS Immunopathogenesis Unit of the San Raffael Medical Center. Dr. Stephano Menzo from Ancona, Italy joined the laboratory for an 8-month sabbatical. Dr. Peter Bressler left the LIR after 10 years to join the faculty at Duke University Medical Center. Dr. Andrew Dayton will leave the LIR at the end of the 1994 fiscal year to assume a position with FDA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00700-01 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Virologic and Immunologic Events Associated with Primary HIV Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	A.S. Fauci	Chief	LIR, NIAID
	C. Graziosi	Visiting Scientist	LIR, NIAID
	M. Vaccarezza	Visiting Fellow	LIR, NIAID
	O. Cohen	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

IRCM, Montreal (R. Sekaly, H. Soudeyns, F. Denis); PRI, Frederick, MD (J. Adelsberger); Univ Alabama/Birmingham (G. Shaw, M. Saag); Tulane Univ (L. Martin); Molecular Histolabs, Gaithersburg, MD (C. Fox); DAIDS/NIAID (C. Dieffenbach, R. Black, P. Sager)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have attempted to delineate certain of the immunopathogenic mechanisms of human immunodeficiency virus (HIV) disease by studying the virologic and immunologic events associated with primary HIV infection. We previously demonstrated that extracellular virions are trapped in the follicular dendritic cell (FDC) network of the lymph nodes (LN) of HIV-infected patients during the clinically latent stage of disease when plasma viremia and virus expression are downregulated. In order to determine the temporal relationship between the decrease in plasma viremia, the downregulation of virus expression, and the trapping of virions in FDC, we studied acute primary infection in the simian immunodeficiency virus (SIV) model. Localization of SIV in the form of virus-infected cells in LN of monkeys is detected as early as 7 days post-inoculation. Trapping of virions in the FDC network is initially detected at 14 days and progressively increases over time, whereas the number of SIV-infected cells decrease. Trapping of virions is temporally associated with a rise in levels of complement-fixing antibodies. These studies indicate that the LN are the sites of establishment of SIV/HIV infection and that virus trapping in LN may be critical to the propagation of SIV/HIV disease. With regard to the immunological events associated with primary HIV infection, we studied six patients soon after primary HIV infection and into the early chronic stage of disease. We demonstrated major expansions in $CD8^+$ T cells that belong to restricted sets of Vß families. These expansions coincided with the peak in viremia and decreased as virus was cleared. The vast majority of the expanded cell populations were activated and they mediated HIV-specific cytotoxicity. Nucleotide sequences of recombinant clones of the expanded Vßs demonstrated the oligoclonal (antigen-specific) nature of these expansions. These expansions of $CD8^+$ T cells expressing a particular Vß family represent an important component of the primary immune response to HIV and should shed insight on the pathogenic mechanisms of HIV disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00676-02 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenic Mechanisms of HIV infection: The Role of Cellular Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	G. Pantaleo	Visiting Scientist	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	C. Muro-Cacho	Visiting Associate	LIR, NIAID
	M. Vaccarezza	Visiting Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

George Washington Univ (J. Orenstein); Molecular Histolabs, Gaithersburg, MD (C. Fox); PRI, Frederick, MD (M. Baseler); Tulane Univ (L. Martin); DAIDS/NIAID (C. Dieffenbach, R. Black, P. Sager)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have investigated the immunopathogenic mechanisms responsible for $CD4^+$ T-cell depletion. Apoptosis intensity is 3- to 4-fold higher in lymph nodes of HIV-infected individuals compared to those of HIV-uninfected individuals. Generally, all compartments of the lymph node are involved in the apoptosis phenomenon in HIV-infected lymph nodes. Apoptosis correlates with the degree of activation of the lymphoid tissue and the chronic immune activation associated with HIV infection. In late stages of HIV disease, apoptosis occurs with similar intensity but in different compartments. Apoptosis does not correlate with progression of the disease (i.e., $CD4^+$ T-cell count) or with viral burden. All lymphocyte subsets (B and T cells, $CD4^+$ and $CD8^+$) have been shown to undergo apoptosis. The importance of cell activation in HIV infection is underscored by the observation that cyclosporin A (CsA), inhibits HIV infection *in vitro* (60% to 80%). CsA has been shown to modulate a series of parameters during primary infection in SIV-infected monkeys. CsA administration delays onset of viremia, prevents a drop in $CD4^+$ T cells and an increase in $CD8^+$ T lymphocytes, increases antibody response, and decreases apoptosis intensity in lymph node.

FUSE 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00585-05 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenic Mechanisms in HIV and Other Retroviral Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.I. Cohen	Medical Officer	LIR, NIAID
Others:	H.C. Lane	Section Head	LIR, NIAID
	V. Kolesnitchenko	Visiting Fellow	LIR, NIAID
	H. Tian	Guest Worker	LIR, NIAID
	R. Lempicki	Staff Fellow	LIR, NIAID
	G. Scully	Clinical Associate	LIR, NIAID

COOPERATING UNITS *(if any)*

CBMB/NICHD (L. Samelson); BRMP/NCI (J. Ashwell); Univ Alabama/Birmingham (G. Shaw); Scripps Institute (P. Russell, C. McGowan); Georgetown Univ (D.P. Hartmann, J. Cossman); Univ California/San Diego (S. Hedrick, J. Newport).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	2.2	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Understanding the molecular mechanisms used by human immunodeficiency virus type 1 (HIV-1) to kill CD4$^+$ T lymphocytes should contribute to finding new therapies capable of interrupting the progression to acquired immunodeficiency syndrome (AIDS). Studies with peripheral blood T cells infected with primary clinical isolates of HIV-1 have shown that HIV-1 kills T cells by trapping the cells at a late point in the cell cycle (G2/M interface). These activated but arrested proliferating cells die when they are unable to complete the cell cycle and an abnormal form of programmed cell death is initiated. The characteristics of this programmed cell death, which include accumulation of proteins found only in cells about to enter mitosis, define the killing event as a pre-mitotic catastrophe. These studies are now being performed on T cells and lymph nodes from HIV-infected individuals 1) to identify populations of T cells in infected individuals undergoing this killing process and 2) to correlate the percentage of affected cells with disease progression to AIDS. We further demonstrated with a panel of metabolic inhibitors that HIV-mediated programmed cell death differed substantially from the programmed death of CD4$^+$ T cells which occurs during normal T cell development (negative selection). These observations have thereby increased the likelihood that clinically useful agents can be developed to selectively inhibit the HIV-killing process without initiating unacceptable toxicity in normal T cells.

FUSE 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00677-02 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Cytokines In the Regulation of HIV Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	A. Kinter	Biologist	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	P. Biswas	Guest Researcher	LIR, NIAID
	D. Weissman	Clinical Associate	LIR, NIAID
	L. Fox	Clinical Associate	LIR, NIAID
	D. Goletti	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

LID/NIAID (V. Hirsch); George Washington Univ (J.M. Orenstein); CABM/UMDNJ, RWMS (A.B. Rabson); Molecular Histolabs, Gaithersbug, MD (C. Fox)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cellular and molecular pathways involved in the regulatory effects of proinflammatory and immunoregulatory cytokines on human immunodeficiency virus (HIV) expression/replication were investigated. The complex interactions between various cytokines, such as interleukin (IL)-10 and tumor necrosis factor (TNF)-α or IL-6, and between cytokines and other cell-mediated signals, such as inducers of cellular differentiation, with regard to regulation of HIV expression were demonstrated in primary monocyte-derived macrophages (MDM) and/or in chronically infected cell lines. Investigation of the molecular basis for these effects demonstrated multiple mechanisms, including transcriptional and post-transcriptional events, whereby cellular factors influence HIV expression. Autocrine/paracrine stimulation of HIV expression by endogenous cytokines was demonstrated in MDM and primary peripheral blood mononuclear cells. In these systems, inhibition of cytokine secretion and/or activity by physiologic agents, such as IL-10, soluble cytokine receptors or antagonists, or by pharmacologic agents, such as pentoxifylline, resulted in significant suppression of HIV replication. Pentoxifylline was also tested *in vivo* in the simian immunodeficiency virus (SIV)-infected macaque animal model. Although no inhibition of acute phase-related TNF-α production was observed, pentoxifylline-treated animals exhibited lower plasma and cell-associated viremia, lower apoptosis in the lymph node, and possibly delayed disease progression. Novel mechanisms of cell-mediated regulation of HIV expression/replication were demonstrated using PBMC or lymph node (LN) cells from HIV-infected individuals or in chronically infected cell lines. IL-12 was found to allow isolation of HIV from unfractionated PBMC or LN cells in the presence of CD8-mediated viral suppressive activity; preliminary data suggest that IL-12 can interfere or bypass CD8-mediated suppression. Triggering of the CD30 molecule, a newly described member of the TNF-α receptor family, was found to potently induce HIV expression in chronically infected cells, most likely by activating the cellular transcription factor NF-κB. These studies indicate that endogenous cytokines may play a major role in the pathogenesis of HIV disease. Such information may prove useful in the design of therapeutic strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00699-01 LIR

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytokine Expression in HIV Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	C. Graziosi	Visiting Scientist	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	G. Pantaleo	Visiting Scientist	LIR, NIAID
	O.J. Cohen	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

IRCM, Montreal (R.P. Sekaly, J.P. Fortin)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We had previously demonstrated the phenomenon and the mechanisms of the regulation of human immunodeficiency virus (HIV) expression by endogenous cytokines. In addition, we had previously determined that the lymphoid organs are the major sites of HIV burden and expression at all stages of disease. We currently have examined the constitutive expression of a panel of cytokines (interleukin [IL]-1ß, tumor necrosis factor [TNF-α], IL-6, IL-2, interferon [IFN]-γ, IL-4, and IL-10) in the peripheral blood (PB) and lymph node (LN) mononuclear cells (MC) of HIV-infected individuals at various stages of disease. We demonstrated that TNF-α, IL-6, IFN-γ and IL-10 were hyper-expressed in the LNMC and PBMC of HIV-infected compared to uninfected individuals; IL-1ß was overexpressed only in the PBMC. These observations are likely to be a reflection of the state of heightened immune activation in HIV infection. Since several of these cytokines have been demonstrated to induce HIV, this over-expression may play a role in the persistent induction of HIV in the milieu of the LN that increases with disease progression. Despite the state of heightened immune activation, IL-2 and IL-4 were poorly expressed at all stages of disease. This dysregulation of cytokine expression may contribute to the immune defects in HIV disease. Contrary to previous reports, in studies involving cross-sectional (among different patients) and longitudinal (within individual patients) analyses, we did not observe a "switch" in predominance from Th-1 (IL-2, IFN-γ) to Th-2 (IL-4, IL-10) patterns of constitutive cytokine expression as disease progressed. Pattern switches were also not observed upon stimulation of cells. In addition, studies on sorted populations of $CD4^+$, $CD4^-$, $CD8^+$, and $CD8^-$ cell subsets demonstrated that even early in the course of disease $CD4^+$ T cell subsets constitutively express very low levels of cytokines. The increased expression of IL-10 and IFN-γ was found largely in the $CD8^{+-}$T cell subsets and among B cells and monocytes. These studies may add insight into our understanding of the immunopathogenesis of HIV disease and may provide potential targets for therapeutic intervention.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00678-02 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Infection of Bone-Marrow and Thymus in the Pathogenesis of HIV Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.K. Stanley	Senior Clinical Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	M.E. Granberry	Staff Fellow	LIR, NIAID
	S. Lee	Medical Staff Fellow	LIR, NIAID
	C. Muro-Cacho	Visiting Associate	LIR, NIAID

COOPERATING UNITS *(if any)*

Molecular Histolabs, Gaithersburg, MD (C. Fox); George Washington Univ (J. Orenstein); PRI, Frederick, MD (M. Baseler); Duke Univ (B. Haynes); ACB/NIAID (P. Golway)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed at delineating the pathogenesis of human immunodeficiency virus (HIV) infection in thymus and bone marrow and developing mechanisms to reconstitute the damaged immune system. We have identified *in vivo* and *in vitro* infection of $CD34^+$ bone marrow progenitor cells. Human thymus is reproducibly infected with HIV in the SCID-hu mouse using primary isolates and is associated with infection of CD4/CD8 double positive, CD4 single positive and CD8 single positive thymocytes. Thymocyte depletion is marked and is associated with increased apoptosis within the thymus. Thymic epithelial cells are also productively infected with HIV and may serve as a viral reservoir in the thymus since they do not appear to undergo cytolysis in response to infection. Other cytopathic effects are observed in the thymus unrelated to direct HIV infection and may represent alterations in growth factor and cytokine production. The SCID-hu mouse provides a model for study of HIV-related superantigen effects which may play a role in the depletion of T cells in infected individuals. We have also shown that cyclosporin A can block HIV infection in the SCID-hu thymus and this may be related to a decrease in cellular activation. Human fetal lymph node and intestine can be infected with intravenously inoculated HIV after implantation in the SCID mouse but further improvements in transplant survival are needed. Reconstitution of the SCID mouse with multiple human tissues (thymus, liver, lymph node, intestine and bone) results in a transient circulating human immune system. These tissues can be infected by intravenous inoculation of HIV and this may provide a model for studying the human immune system in a small animal. $CD8^+$ T cells from certain HIV-infected individuals produce a soluble factor which can suppress HIV expression in $CD4^+$ T cells. $CD8^+$ T cells from various HIV-infected individuals have been immortalized in order to facilitate isolation and characterization of the soluble factor.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00703-01 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Dendritic Cells in HIV Pathogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Weissman	Senior Staff Fellow	LIR, NIAID
others:	A.S. Fauci	Chief	LIR, NIAID
	Y. Li	Visiting associate	LIR, NIAID
	J. Ananworanich	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

Duke Univ (T.F. Tedder, L.-J. Zhou); George Washington Univ (J. Orenstein); Molecular Histolabs, Gaithersburg, MD (C. Fox); PRI, Frederick, MD (M. Baseler)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have isolated three populations of cells with dendritic morphology or the ability to develop this morphology in peripheral blood. Two of the populations appear to be similar cells at different stages of differentiation, both of which can express the dendritic cell (DC) antigen, HB15, while the third population differs in cell surface markers and function. This third population is easilly infectable with human immunodeficiency virus (HIV) while the other cells are not infectable even though all three populations express CD4. The HB15$^+$ DC are able to bind HIV to their surfaces and when mixed with unstimulated CD4$^+$ T cells transfer this virus and initiate an active infection. This infection appears dependent on endogenous cytokines and can be modulated by exogenous cytokines or cytokine modulators. Our data suggest a model for the initiation of HIV infection where DC bind virus, transport it to lymphoid areas, and through activating T cells, initiate a productive infection. Understanding the early events in HIV infection may allow us to develop therapies that may interfere at the earliest stages of infection prior to significant damage to the immune system.

FUSE 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00675-02 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of HIV-Infected Long-term Non-Progressors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.S. Fauci	Chief	LIR, NIAID
Others:	G. Pantaleo	Visiting Scientist	LIR, NIAID
	S. Menzo	Guest Researcher	LIR, NIAID
	M. Vaccarezza	Visiting Fellow	LIR, NIAID
	C. Muro-Cacho	Visiting Associate	LIR, NIAID
	C. Graziosi	Visiting Scientist	LIR, NIAID
	O.C. Cohen	Medical Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

Multicenter AIDS Cohort Study (MACS) Group; Duke Univ (D. Montefiori); George Washington Univ (J. Orenstein); Molecular Histolabs, Gaithersburg, MD (C. Fox).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied a group (n=11) of HIV-infected individuals termed long-term non-progressors (LTNPs) whose disease has not progressed over periods ranging from 7 to 17 years. Levels of viral burden and virus replication were quite low in peripheral blood (PB) and lymph node (LN) mononuclear cells (MC) of LTNPs compared to progressors. Of interest was the fact that relatively high levels of plasma viremia were noted in eight of 11 LTNPs, comparable to levels in HIV-infected individuals whose disease had progressed. The degree of follicular hyperplasia and the total nodal and germinal center areas were significantly less in LTNPs. Of note, and in striking contrast to progressors, the lymph node architecture of the LTNPs were preserved despite several years of infection. Variable degrees of virus trapping was detected; several patients had little if any trapping of extracellular virions. Virus particles were virtually never detected in tissue or cell suspensions by electron microscopy. Only rarely were individual cells detected that were expressing HIV. The lack of virus trapping could in part explain the fact that although only very few cells were actively producing virus in LN, plasma viremia seemed not to be efficiently cleared leading to relatively high levels of plasma viremia. These data together with studies of lymphoid tissue in progressors suggests that disease progression is at least in part related to the deposition of virions on the follicular dendritic cell network of LN germinal centers, persistent activation of LN, active virus replication, and progressive destruction of lymphoid tissue. From an immunological standpoint, HIV-specific cytotoxicity against gag proteins was consistently observed in PBMC of LTNPs. Proliferative responses to a variety of stimuli (mitogens, alloantigens, and recall antigens) were preserved in PBMC of LTNPs. Characterization of humoral immune responses is currently underway. Intensive study of LTNPs should shed valuable insight into the pathogenic mechanisms of HIV disease and hopefully will provide important information for the development of therapeutic and vaccine strategies.

S 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00702-01 LIR

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effect of HIV Infection on Gene Expression in $CD4^+$ T Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	S.M. Bende	Staff Fellow	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	M.E. Granberry	Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have begun to catalogue the differences in gene expression between human immunodeficiency virus (HIV)-infected cells (ACH-2) and uninfected cells (A3.01) to dissect, at the molecular level, the mechanism of HIV-induced immune dysregulation. Using mRNA differential display, we have identified 40 to 50 gene fragments whose regulation in $CD4^+$ T cells appears to be modulated by infection with HIV. Several classes of expression were noted: genes expressed only in infected or uninfected cells, genes up- or down-regulated upon treatment with tumor necrosis factor-α (TNF-α) and, within this latter group, genes whose TNF-α stimulation is infection modulated. We have used similar technology to identify six genes whose expression in primary $CD8^+$ T cells is altered during progression from clinical latency to acquired immunodeficiency syndrome. Ongoing work is aimed at verification/analysis of apparently affected genes to ascertain their role in HIV pathogenesis/immune dysregulation. In addition, we hope to continue to exploit these technologies to better understand the effects of HIV on various cell types, including primary T cells ($CD4^+$ and $CD8^+$) and acutely-infected cells.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00587-05 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gene Expression of the Human Immunodeficiency Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.I. Dayton	Senior Staff Fellow	LIR, NIAID
Others:	E.T. Dayton	Staff Fellow	LIR, NIAID
	D.M. Powell	Graduate Student	LIR, NIAID
	R.K. Hsu	Summer Student	LIR, NIAID
	S. Lee	Clinical Associate	LIR, NIAID
	S. Bende	IRTA Fellow	LIR, NIAID
	L. Butini	Visiting Scientist	LIR, NIAID
	G. Pantaleo	Visiting Scientist	LIR, NIAID
	M.J. Zhang	Visiting Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

LMP/NCI (J.V. Maizel, B. Shapiro); Univ Colorado/Boulder (D. Konings); OD/NIH (P.T. Wingfield, S.J. Stahl); LTCB/NCI (R. Gallo, M. Klotman); James N. Gamble Institute Inst, Cincinnati, OH (B. Baroudy, G. Kotwal)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have identified proteins that specifically bind the effector domain of Rev while it is bound to the Rev response element (RRE). These proteins are being excised from SDS gels for sequencing and cloning. Using similar, but much smaller scale binding technologies, we have developed an *in vitro* binding assay based on the cooperative interaction of the HIV-1 Rev protein, its target sequence, the RRE and previously unidentified cellular factors. Using this assay we have detected in crude extracts a nuclear activity that cooperatively binds with the Rev protein to the RRE. This activity should be crucial for the *in vivo* function of Rev, the emergence from microbiological latency, and the synthesis of mature virions during acute infection. Chromatographic analysis of the activity has determined it consists of at least four and possibly more than five separate proteins. One of the activities has been purified to the extent of five prominent bands on an SDS gel. We should soon be able to sequence and clone it. We have acquired sufficient physicochemical data about the other factors to facilitate their rapid cloning. We are now studying the factors by microinjecting them into mammalian cells along with Rev-dependent indicator genes. In related work we have studied the interaction of the Rev protein with the RRE in the absence of other proteins. We have found that RRE elements determine the binding of Rev molecules subsequent to the first binding event and that Rev must dimerize before binding the RRE. Along slightly different lines, we have used subtractive cloning techniques to isolate clones of a number of cellular genes specifically up- and down-regulated by HIV infection. Surprisingly, HIV infection turns on Line-1, an endogenous human retrovirus-like element, and switches off expression of S16 ribosomal protein. As an extension of our previous work on cell-type-specific viral tropism and the RRE, we have identified other viral determinants of tropism, particularly *vpu* and *vif*. During the course of these studies we have discovered a novel interaction between the *vpu* and *vif* genes.

FUSE 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00701-01 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Therapy on HIV Viral Burden and Replication in Lymphoid Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	O. Cohen	Senior Staff Fellow	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	G. Pantaleo	Visiting Scientist	LIR, NIAID
	C. Graziosi	Visiting Scientist	LIR, NIAID
	M. Vaccarezza	Visiting Fellow	LIR, NIAID
	C. Muro-Cacho	Visiting Associate	LIR, NIAID

COOPERATING UNITS *(if any)*

George Washington Univ (J. Orenstein); Molecular Histolabs (C. Fox); PRI, Frederick, MD (G. Pavlakis); NCI (D. Schwartzentruber); DAIDS/NIAID (S. Schnittman, M. Niu); Palo Alto VA (M. Holodniy); Univ Oregon (M. Loveless); IPL, Carlsbad, CA (R. Moss)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously demonstrated that lymphoid organs are the major reservoir *in vivo* of human immunodeficiency virus type 1 (HIV-1). In early stages of HIV disease, follicular hyperplasia is the predominant histopathologic abnormality seen in lymph nodes (LN). HIV viral particles are trapped efficiently in the follicular dendritic cell (FDC) network. Viral burden and virus expression in peripheral blood (PB) are low during this period. In early stage disease, viral burden and replication are higher (5- to 10-fold) in LN compared to PB. As HIV disease progresses, LN architecture is progressively disrupted and the most common histopathologic abnormalities are follicular involution and lymphocyte depletion. Coincident with FDC network disruption is a loss of viral trapping capacity in germinal centers, increases in viral burden, and replication in PB and LN. The increases in the PB compartment are relatively greater than in LN, thus leading to a decrease in the dichotomy in viral burden, and replication between PB and LN. The effect of standard antiretroviral therapy on HIV burden and expression has been evaluated by sampling PB and LT of patients before and 8 weeks after either remaining untreated, remaining on zidovudine (ZDV), initiating ZDV, or adding didanosine (ddI) to ZDV. In individuals who did not undergo a change in therapy, patterns of histopathology, viral trapping, viral burden, and viral replication remained remarkably constant between week 0 and week 8. In the group that added ddI to ZDV therapy, modest treatment effects (i.e., downregulation of viremia and of levels of virus replication in lymph nodes) were seen. Similar studies are underway or planned to evaluate possible treatment effects of ZDV in later-stage patients. These include studies of dinitrochlorobenzene (DNCB), an immunomodulator, in intermediate-to-late stage patients; and HIV Immunogen in early stage patients.

S 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00390-11 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Anti-Retroviral Therapy of Human Immunodeficiency Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Polis	Senior Investigator	LIR, NIAID
Others:	H.C. Lane	Section Head	LIR, NIAID
	R.T. Davey	Senior Investigator	LIR, NIAID
	R.E. Walker	Senior Investigator	LIR, NIAID
	J. Falloon	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

CC/NIH (H. Masur, J.A. Kovacs); Chiron Corporation (M. Urdea, J. Kolberg, S. Eastman); PRI, Frederick, MD (N. Salzman, R. Dewar, M. Baseler, V. Natarayan)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	1.5	3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed toward examining the relationships between changes in viral burden, changes in viral genotype and phenotype, and changes in immunologic function in patients with human immunodeficiency virus (HIV) infection receiving a variety of anti-retroviral agents with different mechanisms of action alone or in combination. Isolates obtained from patients receiving zidovudine (ZDV) alone were found to develop ZDV resistance sooner than those from patients receiving ZDV in combination with interferon-alpha (IFN-α). In addition, the emergence of resistance was associated with a precipitous increase in the rate of CD4 decline. Neither ZDV nor IFN-α resistance were noted in patients receiving IFN-α alone. Studies of the nucleotide phosphonomethoxyethyl adenine (PMEA) and the immunotoxin CD4-Pseudomonas exotoxin (CD4-PE) were conducted for the first time in humans. Wild-type isolates were noted to have varying degrees of resistance to PMEA. PMEA resistance was mapped to amino acid 65 of the RT gene and found to confer cross-resistance to ddI. While active *in vivo*, PMEA therapy was associated with hepatic and genitourinary tract toxicities. In contrast, no activity was seen in the context of CD4-PE administration. A randomized, controlled trial of the non-nucleoside L697,661 demonstrated a dose-dependant development of resistance within 2 weeks of therapy that was determined to be due to a tyrosine to cysteine mutation at aa 181. New, RNA-based methods were evaluated for the monitoring of viral burden. An infectious clone of HIV underwent an 8-base pair insertion in the region of the LTR. This mutant virus was replication competent and served as an excellent internal control for the measurement of particle-associated RNA in plasma. The branched-DNA (bDNA) technique was found capable of quantitatively measuring changes in viral burden in large numbers of samples to a significantly greater degree than existing techniques. Changes in titers of plasma virus were found to fall within hours of initiation of anti-retroviral therapy and, utilizing a combination of PCR and bDNA, responses to combination anti-retroviral therapy with ddI, ZDV, and the non-nucleoside inhibitor U-90,152S were clearly related to the pre-therapy presence of the 215 mutation of the RT gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00635-03 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Trials for the Prevention and Treatment of HIV-Associated Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	J. Falloon	Senior Investigator	LIR, NIAID
Others:	H.C. Lane	Section Head	LIR, NIAID
	R. Davey, Jr.	Senior Investigator	LIR, NIAID
	M. Polis	Senior Investigator	LIR, NIAID
	R. Walker	Senior Investigator	LIR, NIAID
	M. Sneller	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

CC/NIH (H. Masur, J. Kovacs, K. Spooner, B. Stearn, B. Baird, D. O'Neill, G. Kelly, M. Larson, M. Amantea); PRI, Frederick, MD (M. Baseler); DCBDC/NCI (M. Turner)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort directed at improving the prevention and treatment of acquired immunodeficiency syndrome (AIDS)-associated opportunistic infections continues. HPMPC, a nucleotide analog with activity against cytomegalovirus (CMV) was studied, and a safe and possibly effective dose was identified for Phase III trials. CMVIG-IV (Cytogam) was evaluated for possible activity for the prevention of CMV; it did not decrease the ability to culture CMV from body fluids nor did it prevent disease caused by CMV supporting the notion that antibody is of minimal value in the treatment of active CMV infection. A Phase I/II study of sparfloxacin, an investigational quinolone agent, and azithromycin, a new macrolide agent for the treatment of infection caused by M. avium complex, was recently completed and data analysis is ongoing. A Phase I study of levofloxacin, an investigational quinolone agent that shows promise for the treatment of drug-resistant tuberculosis is almost complete. Enrollment in a study of interleukin-2 (IL-2) for the treatment of mycobacterial infection has begun. Studies of atovaquone, an agent with activity against Pneumocystis carinii, included completion of an assessment of the pharmacokinetics of the licensed tablet formulation of the drug and an assessment of the pharmacokinetics of an investigational suspension formulation of the drug; this suspension formulation was shown to be better absorbed than the tablet. In addition, a study of the efficacy and pharmacokinetics of the investigational suspension formulation of the drug for the treatment of pneumocystis pneumonia has just been completed, and data analysis is ongoing. The pharmacokinetics of dapsone and pyrimethamine were examined by using a population pharmacokinetic model. The assessment and follow-up of persons with idiopathic CD4+ T-lymphocytopenia (ICL) is continuing. Finally, a new outreach effort for the enrollment of women and members of minority and under-served populations in clinical trials has begun, which includes seeing patients in a local community health center. In addition, a major Request for Proposal has been issued to establish collaborations with minority institutions to assist Division of Intramural Research investigators in performing basic and clinical research studies related to *M. tuberculosis*.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00636-03 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Approaches to the Therapy of HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Walker	Senior Investigator	LIR, NIAID
Others:	H.C. Lane	Section Chief	LIR, NIAID
	R.T. Davey, Jr.	Senior Investigator	LIR, NIAID
	J. Falloon	Senior Investigator	LIR, NIAID
	M. Polis	Senior Investigator	LIR, NIAID

COOPERATING UNITS *(if any)*

CC/NIH (H. Masur, J. Kovacs); NIAID (S. Schnittman); MedImmune, Gaithersburg, MD (S. Koenig); Scripps Institute, La Jolla, CA (D. Burton); Chiron Corporation (G. Fyfe); NCHGR/NIH (M. Blaese); CC/NIH (C. Carter, S. Leitvin)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	1.1	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed toward studying the potential therapeutic aspects of immunologic approaches to human immunodeficiency virus (HIV) infection. A study evaluating the immunologic and antiviral effects of repeated administration of interleukin-2 (IL-2) and nucleoside analogs was continued. IL-2 therapy resulted in sustained increases in numbers of CD4 cells and decreased expression of CD8 activation markers; the probability of manifesting these immunologic responses was shown to be directly associated with baseline CD4 count. Using a new plasma HIV RNA detection assay, transient and consistent increases in viral load at the end of each infusion were revealed. In follow-up of these findings, an *in vitro* model of IL-2-induction of viral replication in peripheral blood mononuclear cells was developed and investigations into the mechanisms of IL-2 induction of HIV were begun. A study of IL-2 in patients with Kaposi's sarcoma was initiated, in order to attempt to correlate immunologic and clinical responses. A randomized controlled trial comparing IL-2 plus nucleoside analogs to nucleosides alone was continued, and a randomized controlled multicenter trial comparing 3, 4, and 5 day infusions of IL-2 plus nucleosides to nucleosides alone was initiated. A dose escalation trial evaluating the safety and immunologic activity of subcutaneously administered IL-2 was initiated and an MTD was defined. Toxicities of IL-2 administered subcutaneously were identical to those seen with intravenous IL-2, but generally less severe. Adoptive immunotherapy using transfers of activated and expanded syngeneic polyclonal lymphocytes was completed. Marked but transient increases in CD4 counts were seen in the post-transfer period, often in association with increased viral replication in plasma. CD4 responses were directly related to baseline CD4 count. Studies with gene-marked cells were initiated as a prelude to attempts at gene therapy. A study generating random recombinatorial libraries of human immunoglobulin genes from HIV-infected individuals was continued. An anti-gp120 antibody was identified for clinical development. Studies evaluating the safety and activity of an anti-tumor necrosis factor (TNF-α) antibody and a soluble TNF-α receptor were initiated. Inhibition of circulating TNF-α was not associated with short-term toxicity; no appreciable immunologic or virologic benefit was seen.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00679-02 LIR

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Therapy for HIV Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Bressler	Medical Officer	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	S.K. Stanley	Senior Staff Fellow	LIR, NIAID

COOPERATING UNITS *(if any)*

MHB/NHLBI (R. Morgan)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the past few years, a number of laboratories have become involved in determining the feasibility of utilizing the molecular techniques of gene therapy as part of the strategy for the treatment of human immunodeficiency virus (HIV) infection. One approach has been to transduce into peripheral blood $CD4^+$ T cells selected genes of HIV which have been mutated and which can serve as trans-dominant mutants to block the expression of HIV if that cell ultimately became infected. Ideally, stem cells might be transduced and reinfused into the host thus providing a progeny of cells which are resistent to HIV infection or expression. Another approach to gene therapy has been to transduce into peripheral blood cells antisense HIV genes that when transcribed would bind to HIV mRNA and inhibit the production of HIV. In this regard, we have successfully transduced, using retroviral vectors, cell lines and peripheral blood lymphocytes with genes that express trans-dominant Rev mutants or antisense TAR RNA and have partially protected these cells against spreading HIV-1 infection.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00634-03 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigation of the SCID-hu Mouse Model of HIV-1 Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	R.T. Davey Jr.	Senior Investigator	LIR, NIAID
Others:	H.C. Lane	Section Chief	LIR, NIAID
	M. Boyle	Guest Researcher	LIR, NIAID
	K. Ruxrungtham	Visiting Scientist	LIR, NIAID
	E. Boone	Biologist	LIR, NIAID
	B. Smith	Guest Worker	LIR, NIAID
	M. Flanagan	Guest Worker	LIR, NIAID

COOPERATING UNITS *(if any)*

LMC/NCI (J.S. Driscoll, H. Ford Jr.); MedImmune, Gaithersburg, MD (S. Koenig).

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Clinical and Molecular Retrovirology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	1.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The SCID-hu-PBL model was employed to explore the immunologic and virologic effects of different immune-based therapies on the course of human immunodeficiency virus type-1 (HIV-1) infection as well as to provide a model that could be used to dissect the immunologic aspects of the human immune response to HIV, particularly in patients with stable disease. This model involves reconstitution of 8- to 12-week-old SCID mice with fresh peripheral blood mononuclear cells from human donors. The SCID-hu-PBL model of HIV-1 infection was used to demonstrate that FddA has significant antiviral activity that is consistently superior to ZDV when administered either therapeutically or prophylactically in this experimental system. The adoptive transfer of primed lymphocytes from a gp160-immunized donor coupled with the infusion of high-titer anti-gp160 antibody appears to afford little or no protective immunity against subsequent challenge with HIV-1 in this system. In the latest series of experiments, the model was modified to include direct reconstitution of SCID mice with PBLs from HIV-1-infected donors with stable clinical disease rather than through infection by secondary challenge with purified viral stocks. Intraperitoneal injection of harvested mononuclear cells from infected patients in this hu-HIV/PBL-SCID modification led to successful engraftment in 84% of mice, as confirmed by both PCR detection of human cells and by detection of human Ig antibody production, and caused no diminution in the number of human mononuclear cells harvested by peritoneal lavage relative to control mice engrafted with uninfected cells. Virus was readily recoverable in 98% of engrafted mice using either PCR or co-cultivation methods. Reconstituted hu-HIV/PBL-SCID mice that were untreated sustained a 75% decrease in human $CD4^+$ lymphocyte recovery in peritoneal wash relative to control mice, whereas treatment with the nucleoside analogue FddA both significantly reduced $CD4^+$ cell depletion as well as inhibited viral recovery. Boosting circulating human Ig antibody levels through injection of purified antibody derived from the donor's plasma, or treating mice with a series of human IL-2 injections, did not influence either $CD4^+$ cell survival or viral recovery. In contrast, administration of 10 mg/kg of monoclonal antibody to human tumor necrosis factor-α significantly improved $CD4^+$ cell count survival in reconstituted animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00361-12 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

International Studies on the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T.C. Quinn	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins Univ (A. Rompalo, J. Zenilman, N. Halsey, G. Kelen, R. Bollinger, J. Bartlett)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIH, NIAID, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The acquired immunodeficiency syndrome (AIDS) is a global pandemic with over 14 million human immunodeficiency virus (HIV)-infected individuals worldwide. A major focus within our laboratory has been on defining the unique epidemiologic, clinical, virologic, and immunologic features of HIV-1 and HIV-2 infections in developing countries and in the U.S. In Pune, India, we established a prospective cohort of over 2,000 high-risk individuals attending STD clinics, of whom 25% were seropositive for HIV-1. Over a 4-year time period, seroprevalence rose among commercial sex workers from 7.3% to 44%. Phylogenetic analysis of viral strains isolated from these individuals demonstrates both clade B and C strains with high-titer neutralizing antibody against clade B isolates. In Lagos, Nigeria, we documented a rising seroprevalence of both HIV-1 and HIV-2 among female prostitutes with a strong correlation with genital ulcer disease. Additional studies among STD patients in Baltimore demonstrated the low usage rate of condoms and a high rate of recidivism for STDs. There was a high incidence of HIV infection among these individuals. In perinatal studies of over 500 children born to HIV-infected women in Zaire, Haiti, Malawi, and the U.S., we documented a 28% perinatal transmission rate which correlated with depressed maternal CD4 and elevated CD8 lymphocyte counts, anemia, p24 antigenemia, chorioamnionitis, and funisitis. In addition, perinatal transmission appeared to correlate with elevated concentration of IgG-1 antibodies to the V3 loop in mothers, and with elevated levels of p24 antigen and secretory IgA to gp160 in cervical-vaginal secretions of transmitting mothers. Approximately 3% of infected children may have become infected postnatally from breastfeeding, and virologic studies demonstrated HIV DNA and p24 antigen in 50-70% of breast milk specimens within the first week following delivery. In studies of HTLV infection, we documented a 15.4% perinatal transmission rate of HTLV-1 with an increased risk of transmission in women co-infected with HIV-1. In a prospective study of HIV and HTLV co-infection in Brazil, we documented elevated CD4 lymphocytes, β-2-microglobulin, and p24 antigen level among dually infected individuals. Further studies are planned to elucidate the immunologic modulations of HIV infection by co-infection with HTLV in these cohorts.

FUSE 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00210-14 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulation of Human Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.H. Kehrl	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	K. Druey	Medical Staff Fellow	LIR, NIAID
	V. Kang	Staff Fellow	LIR, NIAID
	A. Riva	Guest Researcher	LIR, NIAID
	C. Thevenin	Visiting Associate	LIR, NIAID
	J. Tuscano	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

BRB/NIAID (J. Coligan); Mount Sinai Med Ctr, NY (V. Najfeld); Georgetown Univ (P. Katz); Harvard Univ (T. Tedder); Toyoma Univ, Japan (Y. Deguchi); Neurologische Klinik und Poliklinik, Germany (P. Rieckmann); PRI, Frederick, MD (J. O'Shea)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

B Cell Molecular Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of the mechanisms that regulate the activation, proliferation, and differentiation of human B lymphocytes. The cytoplasmic domain of the B-cell membrane protein CD22 has been shown to interact with three tyrosine kinases; p53\p56 lyn, p72 syk, and p85 PI-3 kinase via an ARH1 motif. CD22 has been shown to provide a B-cell activation signal and in the presence of cytokines to induce proliferation and Ig production. The interaction of CD22 with its counter receptor on T cells enhances T-cell proliferation. Several new members of the BL34 gene family have been identified. A full-length murine BL34 cDNA has been isolated and a transgenic mouse developed. A fusion protein between the extra-cytoplasmic domain BL11 and the constant region of IgG has been made for functional studies and the identification of a ligand for BL11. The GC kinase protein has been shown to have auto-catalytic activity and a GC kinase antibody localized the protein in germinal center B cells. The majority of the CD20 promoter has been in vivo footprinted. A diverged octamer binding site, a BSAP site, a likely ETS protein binding site, and several sites just proximal to the start sites are protected. Several novel DNA binding proteins have been identified by gel shift assays binding to regions of the CD19 promoter. B-cell lines deficient in CD19 have been isolated, several of which have defective BSAP expression. The CD22 promoters have been in vivo footprinted and several novel cis-elements identified. Promoter constructs have been made and will be useful for mapping the regulatory regions in the promoter. The BTK and BL34 promoters have also been isolated, the RNA start sites mapped, and promoter constructs are in the process of being made. Human genomic clones for HLX and HB9 have been isolated and the nucleotide sequence of each gene determined. The promoters for both genes have been isolated and studies of their regulation initiated. The HB9 gene has been found to be expressed in germinal center B cells and in the GI tract particularily in pancreas. Jak-3 kinase has been found highly expressed in cytoplasmic Ig^+ B cells. Several monoclonal antibodies reactive with activated B cells have been characterized. A study of several transcription factors found in various B-cell subsets has identified several distinctive expression patterns. Bcl-x mRNA transcripts have been analyzed in human B-cell lines and B-cell subsets and found to be low in immature B-cell lines and germinal center B cells.

PHS 6040 (Rev. 5/92)

continued

D. Shaz	HHMI medical student	LIR, NIAID
A. Himmelmann	Guest Researcher	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00431-10 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Molecular Biologic Approach to Immune Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	U. Siebenlist	Section Chief	LIR, NIAID
Others:	K. Brown	Visiting Associate	LIR, NIAID
	G. Franzoso	Visiting Fellow	LIR, NIAID
	T. Kanno	Visiting Associate	LIR, NIAID
	Y.-C. Lin	IRTA Fellow	LIR, NIAID
	M. Tomita-Yamaguchi	Guest Researcher	LIR, NIAID

COOPERATING UNITS *(if any)*

LP/NCI (K. Kelly); Oak Ridge National Laboratory (R. Woychik); FDA (H. Golding); LMDI/NCI (K. Ozato)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immune Activation Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This laboratory has focused on the elucidation of fundamental molecular mechanisms that are involved in immune activation. In particular we have studied the genetic response of mitogenically activated T cells. We cloned by subtraction technology many novel genes induced immediately after stimulation of primary human T cells. They encode transcription factors, cytokines, transmembrane receptors, a nuclear tyrosine phosphatase, and a ras-related protein. Recently we have focused our efforts primarily on NF-κB, one of the transcription factors we cloned. NF-κB is an essential transcriptional regulator of immune/defensive responses. NF-κB is a family of dimeric complexes that are composed of members of the Rel/NF-κB family of polypeptides. NF-κB lies dormant in the cytoplasm of unstimulated cells, kept there by inhibitory IκB proteins. Upon stimulation, NF-κB is released into the nucleus within minutes to potentially induce the transcription of numerous immunomodulatory genes. We have previously determined that appropriate cellular stimulation leads to rapid phosphorylation and proteolytic degradation of IκB, allowing NF-κB to enter nuclei. We also discovered that the activated NF-κB immediately induces its own inhibitor in a negative autoregulatory loop; in this way a transient response is assured. We have now determined that the proteolytic degradation of IκB is an integral part of the activation process, since NF-κB remains associated with IκB even after phosphorylation of the inhibitor. Therefore, IκB proteases provide a potential therapeutic target to block activation of NF-κB in order to control inflammatory conditions. We have begun to define the molecular mechanisms by which the human T lymphotropic virus type 1 Tax product activates NF-κB; the activation of NF-κB is thought to be essential for the transformation of T cells which precedes the onset of adult T cell leukemia. Tax significantly increases the turnover of the IκB inhibitor, and thus weakens cytoplasmic retention of NF-κB. In addition, tax liberates some NF-κB complexes for nuclear translocation by antagonizing the IκB-like cytoplasmic sequestration function of p100. Therefore two separate Tax-mediated mechanisms may contribute to maintain high levels of nuclear NF-κB.

PHS 6040 (Rev. 5/92)

Others continued:

P. Biswas	Visiting Fellow	LIR, NIAID
G. Poli	Visiting Scientist	LIR, NIAID
P. Bressler	Senior Staff Fellow	LIR, NIAID
A.S. Fauci	Chief	LIR, NIAID

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00213-14 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical, Immunopathogenic, Therapeutic Studies of Vasculitis and Other Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	M.C. Sneller	Senior Investigator	LIR, NIAID
Others:	A.S. Fauci	Chief	LIR, NIAID
	G.S. Kerr	Visiting Associate	LIR, NIAID
	C. Talar-Williams	Study Coordinator	LIR, NIAID

COOPERATING UNITS *(if any)*

DCBDC/NCI (W.D. Travis); OSD/NIAID (J.I. Gallin, C. Hallahan)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunologic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Following our previous therapeutic success with cyclophosphamide (CP) in the treatment of Wegener's granulomatosis (WG), we have gone on to evaluate the safety and efficacy of methotrexate (MTX) as an alternative therapy in this disease. Forty-two patients who did not have immediately life-threatening disease were studied. Glomerulonephritis was present in 21/42 (50%) of patients. The median follow-up was 23.4 months. Weekly administration of MTX and prednisone resulted in remission of disease in 30/42 patients (71%). The median time to remission was 4.2 months. Twelve patients did not achieve remission: three patients had progressive disease that required institution of CP therapy; five patients had clinical improvement but continued to exhibit signs of active disease; two patients developed MTX-induced pneumonitis prior to achieving remission; two patients died of opportunistic infections prior to achieving remission. For the 30 patients who achieved remission: 23% relapsed within 1 year of achieving remission and 40% relapsed within 2 years. The estimated median time to relapse for all patients achieving remission was 29 months. In patients with glomerulonephritis at study entry, relapse occurred earlier (35% by 1 year) than in patients without glomerulonephritis (no relapses at 1 year); however, after 2.5 years the estimated percent of patients who had relapsed was similar in the two groups (50% vs 57%; p=0.1). Eight patients who relapsed were treated with a second course of MTX plus prednisone. A second remission was induced in six out of 8 (75%) patients. MTX plus prednisone may be an acceptable alternative form of therapy for selected patients with WG. In preliminary studies we have used skin blisters induced by suction as a method to study the *in vivo* inflammatory response of patients with WG. We have shown that patients with active WG produce 50- to 100-fold higher levels of TNF-α in blister fluid when compared with normal controls. Such high TNF-α levels are not seen in blister fluid from patients with a variety of other inflammatory and infectious diseases. Levels of IL-1ß and IL-6 were also significantly elevated in blister fluid from patients with active WG. These findings have important therapeutic implications since specific inhibitors of TNF-α and other pro-inflammatory cytokines are currently available and may be effective in the treatment of WG and related systemic vasculitides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00358-11 LIR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunopathogenesis of *Chlamydia trachomatis* Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: T.C. Quinn Senior Investigator LIR, NIAID

COOPERATING UNITS *(if any)*

Johns Hopkins Univ (C. Gaydos, L. Bobo, R. Viscidi, S. West, J. Zenilman, A. Rompalo)

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

Immunopathogenesis Section

INSTITUTE AND LOCATION

NIH, NIAID, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in the U.S. with an annual incidence of 5 million cases. Studies have been in progress to define the clinical spectrum of chlamydial infection, to develop improved diagnostic assays, and to examine the pathogenesis of chlamydial infections. In screening 4,000 patients attending STD clinics, we have demonstrated that molecular amplification assays, including polymerase chain reaction (PCR) and ligase chain reaction (LCR) have markedly improved the sensitivity of detection of *C. trachomatis*. In screening urine samples by PCR or LCR, the prevalence of chlamydia infection was as high as 28% in school-based adolescent clinics compared to 15% by genital culture. In transmission studies of 106 sexual partnerships with chlamydial infection, 52% were concordantly PCR positive. In contrast to culture results, there was no significant difference in male-to-female vs. female-to-male transmission, which was estimated to be 68%. Genetic sequence analysis of amplified chlamydial DNA from these partner pairs confirmed the presence of the same serovar, further supporting the accuracy of PCR. Similar results have been obtained by LCR for the detection of *Neisseria gonorrhoeae*. We developed a new serologic technique for the measurement of specific antibodies to the major outer membrane protein (MOMP) referred to as a transcription-translation radioimmunoassay (TT-RIA). Use of this assay demonstrated a serologic rise in specific anti-MOMP antibodies in MOMP-vaccinated primates, which directly correlated with decreased infectious load. Following the development of PCR for *C. pneumoniae*, we have identified *C. pneumoniae* in 8% of 700 patients with respiratory disease. Antimicrobial susceptibility studies have demonstrated the potential efficacy for azithromycin and clarithromycin for treatment of this pathogen. Although we have been able to demonstrate *in vitro* replication of *C. pneumoniae* in human pulmonary macrophages, aortic endothelial cells, and aortic artery smooth muscle cells, we have not been able to demonstrate by culture or PCR *C. pneumoniae* in atheromas of selected patients with coronary heart disease. Additional studies are planned to further address the role of *C. pneumoniae* in respiratory disease and atherosclerotic heart disease.

PHS 6040 (Rev. 5/92)

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